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AUREOMYCIN - A NEW ANTIBIOTIC

by

B. M. DUGGAR, L. ANIGSTEIN, J. BENINSON, E. A. BLISS, A. E. BRALEY, R. L. BRICKHOUSE, M. S. BRYER, C. A. CHANDLER, M. C. CLARK, H. S. COLLINS, R. COSGROVE, H. R. COX, R. W. CUNNINGHAM, A. C. DORNBUSH, H. F. DOWLING, M. FINLAND, B. K. HARNED, C. H. HINE, L. M. HILL, E. H. LENNETTE, M. H. LEPPER, P. A. LITTLE, M. A. LOGAN, P. H. LONG, W. J. McCAULEY, G. MEIKLEJOHN, T. F. PAINE, Jr., E. J. PELCAK, C. W. PRICE, A. PRIGOT, W. A. RANDALL, M. SANDERS, E. B. SCHOENBACH, E. STOKEY, Y. SUBBAROW, L. K. SWEET, H. M. THELEN, R. E. VESSEY, H. WELCH, D. M. WHITNEY, S. C. WONG, L. T. WRIGHT and N. N. YUDA

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AUREOMYCIN – A NEW ANTIBIOTIC*

Consulting Editor: J. H. WILLIAMS

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*This series of papers is the result of a Conference on *Aureomycin* held by the Division of Mycology of the Section of Biology, The New York Academy of Sciences, on July 21, 1948. Publication made possible by a grant from Lederle Laboratories Division, American Cyanamid Company.

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AUREOMYCIN: A PRODUCT OF THE CONTINUING SEARCH FOR NEW ANTIBIOTICS

By B. M. Duggar

Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

THE articles herewith presented discuss a new antibiotic from a new species of the Actinomycetes—these Actinomycetes constituting a group of fungi which might well be called ultra-molds. We are concerned with a species of the established genus *Streptomyces*, for which the name *Streptomyces aureofaciens* is being proposed. The significance of the specific name will soon be apparent. This is a soil organism which seems to have escaped previous publicity and domestication, as well as having evaded a pigeonhole in the accessions of mycological science.

The antibiotic (aureomycin) from this species of mold will be evaluated, in preliminary measure at least, in relation to the role which, at this stage of the studies in progress, it may promise to play safely in the treatment of disease. Academically, the antibiotic itself and the organism producing it are of very considerable interest, whatever may be the score as affecting human health or animal production. But we measure our interest in it, at the present time, primarily by the qualities which it may display as a systemic and an effective chemotherapeutic agent.

It was most fortunate that so soon as reasonable quantities of aureomycin became available and pharmacologically acceptable, it was possible to obtain the cooperation of so many recognized leaders in the fields of experimental, clinical, and other aspects of medicine and medical work to give it immediate hospital trial, thus gaining the weight of their judgments as to its future possibilities. The authors, as well as the titles of their papers, suggest the wealth of skill and experience, the variety of diseases available, and the character of the facilities employed in the observations and investigations which followed.

My purpose is, first, to be introductory but brief; secondly, to say something of the characteristics of the source species and of its antibiosis *in vitro*, especially pointing out or emphasizing the range of disease-inducing or other organisms which, by growth-inhibition (for one criterion), have demonstrated susceptibility to aureomycin, and likewise to suggest certain groups of organisms which are more resistant.

Before proceeding with a very few of the details referred to, permit me to make two acknowledgments. The first is this: In referring to any experimental work with either the proposed *S. aureofaciens*, or with the product aureomycin, in this preliminary record, it should be clear without further emphasis that the direction of the work by the late Dr. SubbaRow and his sustained encouragement are gratefully acknowledged; likewise cordially acknowledged are the suggestions of co-workers, particularly the assistance of my associates, Misses Doris Dansby and Dorothy Evans. The second acknowledgment is of a different order: It is that I shall present today no history of antagonism, antibiosis, and aversion of micro-organisms nor any citation of important events characterizing either the present antibiotic period or the earlier ante-antibiotic period. It may be added that much has been said on antibiotic history in the Academy's conference room, and the record is available in its *Annals.* Nevertheless, there are a few general facts and figures which seem to require rehearsal for better orientation.

From digests and summaries appearing in 1946–47 it appears that, up to that time, there were recognized roughly 30–35 antibiotics, excellent or indifferent, having their origin in the metabolic products of the usual molds or filamentous fungi, and there were about the same number of such agents attributable to the bacteria. In considerable part the two groups of substances are unlike. Then there are the Actinomycetes, which the rank and file of the mycologists treated with static contempt—and the bacteriologists in large part kicked around like the legendary "houn'-dog." These last, the Actinomycetes, furnished, up to the time mentioned, about a dozen antibiotics, and typically these present few products resembling those of the bacteria. The total of these antibiotics or substances listed as such was then about 80.

It is likely that the present total is now more nearly 100, the result of feverishly active surveys and researches during this post-war period. Perhaps half of the recent product "finds" have resulted from studies in the Basidiomycetes, commonly known as fleshy, bracket, or punk fungi. About as many additions come from the Actinomycetes and the bacteria. Of this respectable galaxy, the industrial output of therapeutic agents is largely limited to penicillin and streptomycin, fumigacin and streptothricin among mold products being as it were tagged for further study as systemic agents. The status of the bacterial products is still largely uncertain partly because some are new arrivals, although gramicidin, tyrothricin and bacitracin have, perhaps, an established but limited place.

Few persons, I think, would wish to commit themselves very far on the outlook for the future. The guess might be ventured that the apparent failures to bring forward new antibiotics arising from filamentous molds may be less indicative than the silence seems to warrant. It may be that investigators have become sensitized to high potencies, as witnessed in the stepped-up yield of penicillin. It should not be overlooked that initially low potencies (relatively) have also characterized other products than penicillin. It may be guessed also that fame-seeking Actinomycetes are not in waiting around our doorsteps, and the cost of going after them and the disappointments to be achieved are some things to contemplate. Nevertheless, the promise, I would say, is more than adequate to justify continuance of the search.

Returning now to a limited characterization of the proposed *Streptomyces aureofaciens*, a significant point is this: At a certain stage in the growth of the colony of this fungus, or of a smear (an amalgamation of colonies), there is "typically" the production of a golden yellow pigment in the moist or hygrophorus substrate mycelium, hence the proposed specific name, *aureofaciens*, signifying a golden appearance. Moreover, the antibiotic is also faintly golden yellow, an added reason why this antibiotic from *S. aureofaciens* is designated *Aureomycin.*

Color production is commonly well developed in most strains of this organism isolated from the soil and grown on meat extract-asparagine-dextrose agar or on potato-dextrose agar, and on certain other media as well, notably on potato cylinders. Account is not taken here of certain "washed-out" strains and mutants. Grown on agar, the substrate mycelium of young colonies is practically hyaline at first, commonly becoming yellow in 2–3 days. The aerial or "dry" mycelium is white, that is, without color at first. Likewise, the first-formed spores are white, but the entire heavily sporing surface of a slanted agar culture gradually changes (in 5–7 days at 28° C.) through brownish gray to a dark, drab gray. At the same time most of the substrate mycelial color disappears. The reverse color of slants at its best is golden tan, later tawny. With continued incubation, or if refrigerated, the mycelial color is promptly dulled. Microscopically, the hyphae have the appearance of true fungous filaments, and the aerial ones break up into chains of spores in a manner characteristic of certain fungi.*

There are several species of *Streptomyces* characterized by the production of yellow pigment in the mycelium or in the culture medium—the latter the so-called soluble pigments. With the cooperation of Mr. C. W. Hesseltine a careful comparison has been made with the original description of each of these species and with living culture material so far as available. The following are included under the "yellow" species of *Streptomyces* with which comparisons have been made: *S. flaveolus*, *S. californicus*, *S. cellulosae*, *S. parvus*, *S. flavochromogenes*, *S. antibioticus*, *S. aureus*, *S. flavus*, *S. citreus*, *S. fulvissimus*, *S. alboflavus*, and others which are omitted for various reasons. This study has involved, of course, detailed consideration of those reactions termed "cultural characteristics," as also of certain special biochemical properties, all of which will be presented in the form of a technical diagnosis in a paper now being held for publication, to follow the printing of this report.

The present report would be wholly incomplete without an adequate statement of the *in vitro* antibiosis of this species. Besides many tests of all strains, a typical strain, A-377, has been tested, to show the sensitivity or resistance of each of more than 50 species of the usual or unusual assay organisms. The list of organisms tested includes pathogenic bacteria (human or animal), non-pathogenic bacteria, plant pathogens, and

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*Figures 1, 2, 3, and 13 display the development of *S. aureofaciens* as far as it seems practicable to exhibit this aspect of its life history at the present time.

various filamentous fungi and yeasts. The procedure used was not merely the usual form of spectral test, but rather placements (colonies) of the growing A-377 (or related strains*) were grown for approximately 75 hours on agar plates appropriately flooded with the assay organism, somewhat as in the case of the cup technique. The assay, however, involves the exposure of the flooding organism to the antibiotic directly as secreted by the growing colonies of *S. aureofaciens*. Just as a demonstration of the procedure, refer to FIGURE 10, displaying *S. griseus* colonies (or placements), tested against *Trichophyton mentagrophytes* as the flooding, or assay, organism. This is used because the zones are much smaller than those of our A-377 and the differential areas are defined more nearly in the center of the plate. The diameter in mm. of the zone of inhibition, under standard conditions, furnishes an approximate criterion of activity or potency of the antibiotic-furnishing strain, or conversely an index of sensitivity of the assay species. Eventually the activity of the strain may be compared with a standard. As exhibited on the plate, activity is related to inherent potency and concentration of the antibiotic and its rate of diffusion, as well as to other factors not here considered.

Twenty-two kodachrome slides were made displaying inhibition zone assays, a number of which show "off-plate" or "practically off-plate" effects in relation to the assay organism.† Essentially all isolates, more or less directly from soil dilution cultures gave off-plate, often spectacular values with the following gram-positive bacteria: *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Mycobacterium tuberculosis* (607), and with the following gram-negative ones, *Escherichia coli*, *Aerobacter aerogenes*, *Salmonella pullorum*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Neisseria catarrhalis*, and *Eberthella typhosa*. High values

* The induced mutant A 377–899 was furnished by my colleague, Dr. E. J. Backus.

†These are here represented by FIGURES 4–12 and 14–24.

FIGURES 1–12 (*see opposite page*)

FIGURE 1. Development of colonies of *Streptomyces aureofaciens* (A 377) growing on meat extract-dextrose-asparagine agar.

FIGURE 2. Agar slant culture of *S. aureofaciens* (A 2020) sporing heavily.

FIGURE 3. Close-up of colony of *S. aureofaciens* (A 2020), displaying particularly the early development of white aerial hyphae.

FIGURE 4. Large inhibition zones in assay of zone values of *S. aureofaciens* (isolate A 271) against *Staphylococcus aureus*.

FIGURE 5. Overlapping and off-plate (designated OP values) "zones" of *S. aureofaciens* (isolate A 377) against *Staph. aureus* as assay organism.

FIGURE 6. Practically OP inhibition zones of *Penicillium chrysogenum* (strain Q 176) against *Staph. aureus*.

FIGURE 7. OP inhibition zones of *S. aureofaciens* (isolate A 232) against *Mycobacterium tuberculosis* (No. 607).

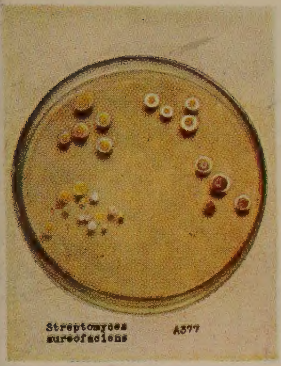
FIGURE 8. Partly overlapping inhibition zones of *S. aureofaciens* (isolate A 1684), an organism of characteristically weak potency in this group of isolates, against *Mycob. tuberculosis* (No. 607).

FIGURE 9. OP inhibition zones of *S. aureofaciens* (A 377) assayed against the fungus *Trichophyton mentagrophytes*. The scattered clumps are flakes of mycelium of the assay organism introduced with the spores, no growth arising from the flakes in 48 hours.

FIGURE 10. Inhibition zones produced by *S. griseus* (strain A 1) against *Trichophyton mentagrophytes*.

FIGURE 11. OP inhibition zones of *S. aureofaciens* (induced mutant A 377–899) against *Salmonella pullorum*.

FIGURE 12. OP inhibition zones of *S. aureofaciens* (induced mutant A 377–899) against the organism *Phytomonas tumefaciens*, the causal bacterial agent of "crown gall" of plants.



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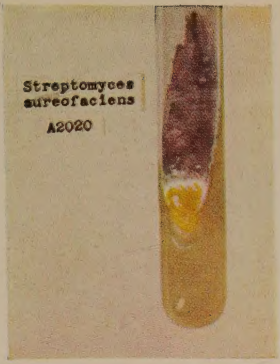

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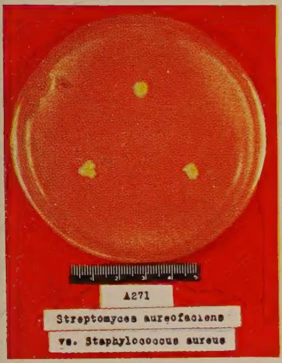

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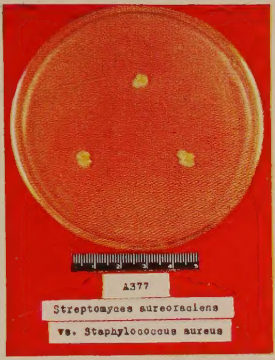

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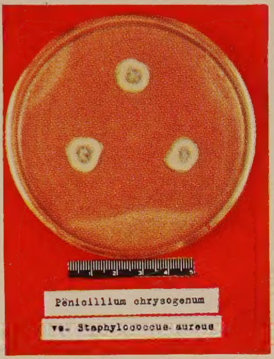

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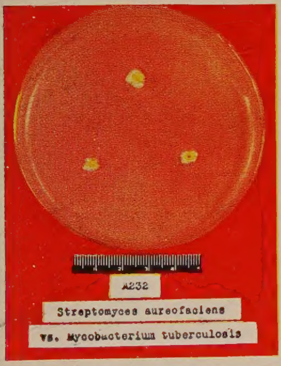

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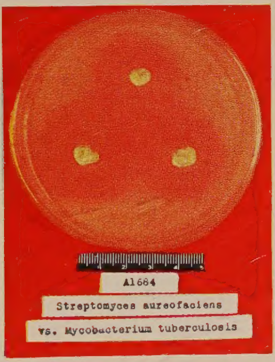

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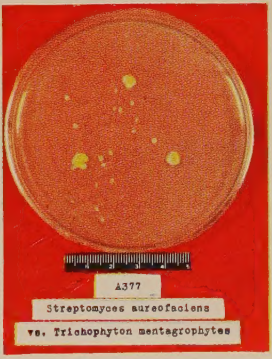

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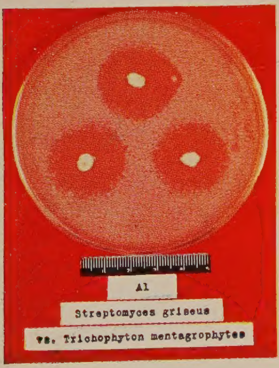

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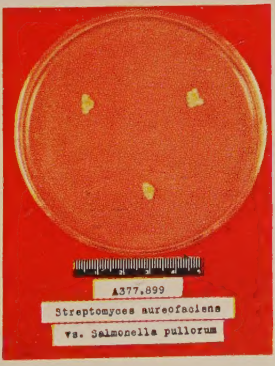

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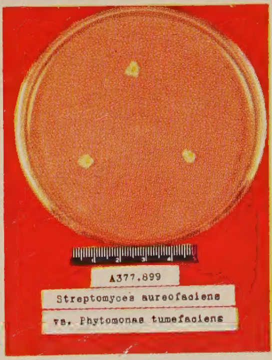

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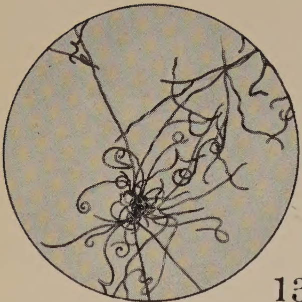

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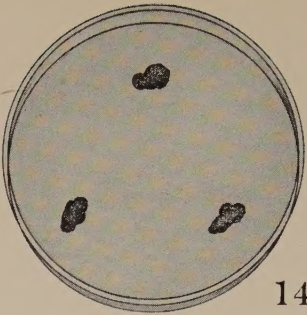

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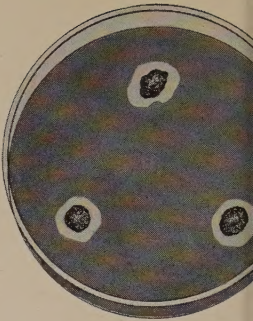

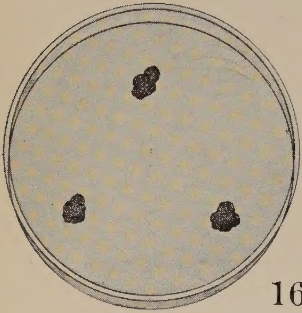

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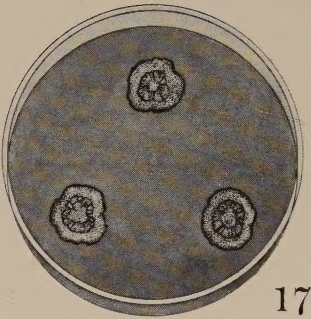

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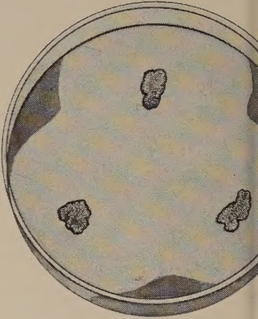

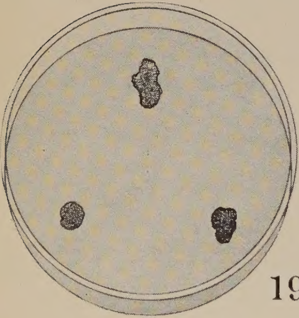

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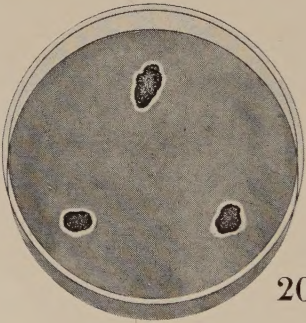

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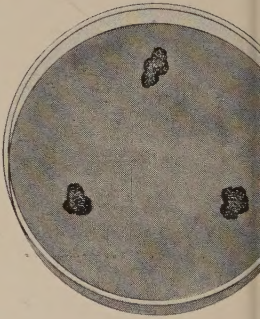

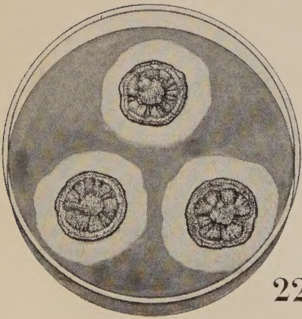

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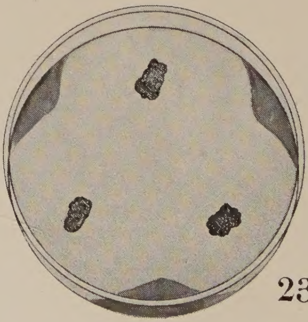

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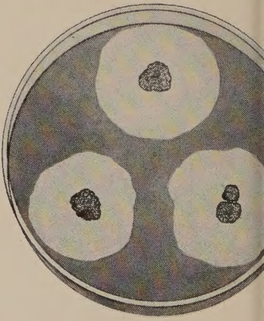

were also obtained against *Shigella gallinarum*, *Brucella abortus* and other species. Relatively resistant were *Pseudomonas aeruginosa* and *Serratia marcescens*, while the majority of the fungi and yeasts are strongly resistant. The figures display differences and relations not mentioned in the text. The photographic work on the original slides was done by Mr. L. H. McWilliam. The paintings, from which four of the slides were prepared, were the work of Mr. A. A. Jansson.

The culture of *Streptomyces aureofaciens* in shaken flasks and tanks has been satisfactory. The highly purified, crystalline product has been tested in broth at a favorable pH against a variety of organisms in order to determine more nearly absolute effects. These values confirm in considerable measure the expectations formulated as a result of the plate-zone tests, but they will not be presented here in view of the probability that other papers today will cover this aspect of the work. Tests made at a pH much above pH 7 have little point, since loss of the product by precipitation introduces one type of error, or, if the period of observation is shortened, the extent of growth, if any, is limited and the turbidimetric assay rendered uncertain.

Now, when all this demonstration of conspicuous activity has been displayed by plate zones and other types of assay, one is certain that there is a potent antibiotic present. It is in this sense only, at that stage, a desirable antibiotic. It must be demonstrated that the organism will give fair to high potencies when cultured in suitable liquid media, and the product obtained must assay accordingly when tested against some of the same microorganisms. The problems of isolation, purification, and crystallization of the antibiotic are at hand. Finally, there are the various types of *in vivo* activity, of stability, pharmacological suitability, and of efficiency for particular infections. These all will be treated in the following papers.

FIGURES 13–24 (*see opposite page*)

It is to be noted that there are three values in the intensity of the tones used in FIGURES 13–24, and the following explanation applies in all cases: (a) the dark tone (ink stipple) represents the fungus tested for antibiotic activity, that is, the "placement" organism present in each of the eleven figures indicated above; (b) the medium dark "wash" represents the assay or flooding organism, always present when the assay organism displays growth; and (c) the light wash indicates inhibition of growth of the assay organism, inhibition zones, and these latter may overlap and thus eliminate any growth of the assay species on the plate.

FIGURE 13. Aerial hyphae of *Streptomyces aureofaciens* (A-377) at the time of spore differentiation.

FIGURE 14. Off-plate (OP) values of inhibition zones of *S. aureofaciens* (A 377–899) against *Bacillus cereus*.

FIGURE 15. Inhibition zones of *S. griseus* against *Staph. aureus*.

FIGURE 16. OP inhibition of *S. aureofaciens* against *Mycobacterium tuberculosis* (No. 607).

FIGURE 17. No zones of inhibition produced by *Penicillium chrysogenum* (Q 176) against *Mycob. tuberculosis* (No. 607).

FIGURE 18. Practically OP values of *S. aureofaciens* against *Coccobacillus* sp., an assay species strongly resistant to streptomycin.

FIGURE 19. OP inhibition of *S. aureofaciens* (A 377–899) against *Escherichia coli*.

FIGURE 20. Essentially no inhibition produced by *S. aureofaciens* against *P. chrysogenum* (Q 176).

FIGURE 21. No zones of inhibition in the test of *S. aureofaciens* (A 377–899) against *Aspergillus niger*.

FIGURE 22. Small zones of inhibition produced by *P. chrysogenum* (Q 176) against *S. aureofaciens* (A 377–899).

FIGURE 23. Practically OP values of *S. aureofaciens* (A 377–899) against *S. griseus* (A 1).

FIGURE 24. Zones of inhibition produced by *S. griseus* against *S. aureofaciens* (A 377–899).

THE PHARMACOLOGY OF DUOMYCIN

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DUOMYCIN,* aureomycin A-377, is a yellow crystalline antibiotic obtained from the recently characterized mold *Streptomyces aureofaciens.*[1] Its potency is unusual both in degree and range in that it is effective against numerous gram negative and gram positive organisms as well as against some rickettsial and viral infections. This activity, coupled with a low toxicity, indicates that it is a potentially valuable chemotherapeutic agent worthy of extensive pharmacological analysis.

Animals and Methods

The acute toxicity of single doses has been studied in mice, rats, guinea pigs, rabbits, cats and dogs. The mortality count was made 72 hours after the dose was administered. The subacute toxicity from multiple doses has been studied in mice and dogs and the chronic toxicity in mice, rats, dogs and chickens. Including the pharmacodynamic studies, the problem has required 46 dogs, 24 cats, 36 rabbits, 109 guinea pigs, 559 rats, 760 mice and 18 chickens.

Electrocardiographic studies were made with a Cardiotron on anesthetized and unanesthetized dogs. Blood pressures were recorded in unanesthetized dogs by femoral arterial puncture and in anesthetized animals by cannulation of the carotid artery. Hemoglobin was determined as cyanmethemoglobin. The Wendel procedure[2] was used in the examination for methemoglobin. Clotting time was determined by the capillary tube method. Blood sugar was determined by the Shaffer-Somogyi method.[3] The Lipschitz assay[4] was used to evaluate diuretic activity.

Esbach's reagent was used in the tests for albuminuria. The filtered urines were treated in calibrated 15 cc. centrifuge tubes with the picric acid reagent, centrifuged, and compared with standardized serum albumin solutions treated similarly.

Phenolsulfonphthalein was used in the renal function tests in dogs. The routine was as follows: (a) Food was withheld from these animals for 24 hours; (b) fifteen cc. of water per kilogram of body weight was administered orally and, thirty minutes later, the bladder was drained and washed with 0.9 per cent sodium chloride; (c) the mono-sodium salt of phenolsulfonphthalein was injected intravenously in a dose of 0.4 mg.

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* Trade mark.

per kg.; (d) at one hour and at two hours, the dogs were catheterized and the bladders washed with 0.9 per cent sodium chloride; (e) the specimens were alkalinized and the amount of dye determined with a photoelectric colorimeter (filter No. 540). Normal dogs excreted from 60 to 85 per cent during the first hour.

Liver function was estimated by injecting intravenously 5 mg. per kg. of bromsulphalein. Blood was drawn at 5 and 30 minutes subsequent to the dose and the amount of the dye retained in the serum was estimated with a photoelectric colorimeter (filter No. 560). Normal retention at thirty minutes was from 0 to 15 per cent.

Tests for histaminic action were made on isolated guinea pig gut and also by following the changes in blood pressure in anesthetized cats. Antihistaminic action was tested on the isolated guinea pig gut and on guinea pigs in a spray chamber.

Tests for antipyretic action were made by following the rectal temperatures of rabbits injected intravenously with 0.25 cc. per kg. of typhoid vaccine, and of rats injected subcutaneously with 10 cc. per kg. of a 15 per cent suspension of yeast.[5]

Tests for irritation were made by the intracutaneous injection into the guinea pig skin and by local application to the conjunctival sac of the rabbit eye. In the eye, three or six drops at five minute intervals were used. Blood, urine and cerebrospinal fluid levels of duomycin were estimated biologically against *Bacillus subtilis* using a four hour period.[6]

Duomycin is soluble in acid and in alkaline solutions, but is almost insoluble at pH 7. Four per cent solutions may be easily prepared as the hydrochloride pH 2.5 or as a sodium salt in a carbonate buffer pH 8.5. At pH 2.5, the salt is stable but at pH 8.5, 25° C., it loses 12 per cent of its activity in 30 minutes, 20 per cent in 1 hour, and 40 per cent in 2 hours. Duomycin was administered parenterally usually as a 1 or 2 per cent solution pH 2.5 or pH 8.5. The hydrochloride was always used for the oral doses unless a different form was specified.

Acute Toxicity

Single Doses, Unanesthetized Animals. Orally, the toxicity is of a low order. Mice tolerated 1500 mg. per kg. and rats, 3000 mg. per kg. At 2500 mg. per kg., the mouse-mortality was 5 per cent (TABLE 1). No attempt was made to determine the maximal tolerated dose in larger animals. Intravenously, the L.D.$_{50}$ for the hydrochloride was 134 mg. per kg. for mice and 118 mg. per kg. for rats (FIGURE 1). The alkaline form appeared to be a little more toxic in mice but less toxic in rats (TABLE 1). These differences, however, are not significant. Three unanesthetized dogs readily tolerated intravenously, doses of 50 mg. per kg., pH 2.5,* given at a rate of 10 mg. per kg. per minute. Some days later, this dose in

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* Doses larger than 30 mg. per kg. of duomycin hydrochloride pH 2.5 produce hemoglobinuria. An equivalent quantity of hydrochloric acid will produce the same result. Hemoglobinuria has never been observed after the injection of duomycin pH 8.5, even with doses of 100 mg. per kg.

TABLE 1—ACUTE TOXICITY OF DUOMYCIN

														L.D.50	
														mg./kg.	19/20 Fiducial limits
Oral dose—mg./kg.			1000	1250	1500	1750	2000	2500	3000						
Mice	in 2% Starch pH 2.5	No. Injected	20		20		20	20	20						
		No. Dead	0		0		1	1	7						
Rats	in 2% Starch pH 2.5	No. Injected	15	3	15	3	15	10	10						
		No. Dead	0	0	0	0	0	0	0						
*Intravenous dose—mg./kg.**			40	50	60	70	80	90	100	125	140	150	175		
Mice	Aqueous pH 8.5	No. Injected		11		14			11	10		10	5		
		No. Dead		0		1			4	9		9	5	102	88.7–117.3
	Aqueous pH 2.5	No. Injected	50	50	10	10	90	50	50	50	10	13	13		
		No. Dead	1	0	0	0	1	1	0	24	3	10	11	134	115.5–155.4
Rats	Aqueous pH 2.5	No. Injected		3	2	2	2		10	8	6				
		No. Dead		0	0	0	0		0	5	5			118	103.5–134.5
	Aqueous pH 8.5	No. Injected								10	6				
		No. Dead								1	1				
Guinea Pigs	Aqueous pH 2.5	No. Injected							5	3					
		No. Dead							2	3					
	Aqueous pH 8.5	No. Injected							5						
		No. Dead							2						
Subcutaneous dose—mg./kg.			100	200	300	400	500	600							
Mice	Aqueous pH 2.5	No. Injected	8	8	4	10	10	10							
		No. Dead	0	0	0	2	3	1							
Guinea Pigs	Aqueous pH 8.5	No. Injected		2	3										
		No. Dead		0	0										

* Injection Rate: Mice, 250 to 500 mg./kg./min.; rats, 35 mg./kg./min.; guinea pigs, 50 mg./kg./min.
Weight ranges: Mice—14–25 gms., rats—100–315 gms., guinea pigs—327–580 gms.

TABLE 1-A

THE EFFECTS OF MULTIPLE INTRAVENOUS DOSES OF DUOMYCIN pH 8.5 ON DOGS

Schedule: Blood counts and function tests on Feb. 20, 23, 24 and March 3 and 4; Duomycin doses: Feb. 24, 10 mg./kg. A.M., 20 mg./kg. P.M.
Feb. 25, 26, 27, 20 mg./kg. A. M. & P.M.; Feb. 28, 29, 20 mg./kg. A.M.; March 1, 2, 20 mg./kg. A.M. and P.M.

Dog No.	Weight Feb. 20 (kg.)	Weight Mar. 2 (kg.)	Weight Apr. 4 (kg.)	Bromsulphalein test for liver function: Before Duomycin, Retention 5 min. (per cent)	Before Duomycin, Retention 30 min. (per cent)	After Duomycin, Retention 5 min. (per cent)	After Duomycin, Retention 30 min. (per cent)	Clotting time of blood: Before Duomycin (min.)	After Duomycin (min.)	Phenolsulfonphthalein test For kidney function, Two hour excretion: Before Duomycin (per cent)	After Duomycin (per cent)
0260	7.6	7.1	7.4	16	9	21	11	3.3	3.5	74	91
0257	6.1	5.8	6.8	8	4	14	5	3.3	3.5	71	79
269	7.9	7.3	8.3	28	10	22	6	3.5	4.4	73	73
283	7.0	6.6	7.8	23	6	38	17	3.8	4.9	66	72
249	6.4	6.0	6.7	18	8	16	6	3.4	2.5	64	85
278	6.8	6.3	7.1	35	4	45	10	3.9	3.5	75	93

Blood Counts

Dog No.	Hemoglobin: Before Duomycin (gms./100 cc.)	Hemoglobin: After Duomycin (gms./100 cc.)	Total blood cell count, Red blood cells: Before Duomycin (millions per cu. mm.)	Red blood cells: After Duomycin (millions per cu. mm.)	White blood cells: Before Duomycin (thousands per cu. mm.)	White blood cells: After Duomycin (thousands per cu. mm.)	Neutrophils: Before Duomycin (per cent)	Neutrophils: After Duomycin (per cent)	Eosinophils: Before Duomycin (per cent)	Eosinophils: After Duomycin (per cent)	Lymphocytes: Before Duomycin (per cent)	Lymphocytes: After Duomycin (per cent)
0260*	17.9	15.8	8.13	8.80	12.0	11.1	51	55	15	6	33	38
0257*	13.9	12.6	7.45	6.70	10.9	14.7	48	65	10	7	41	28
269*	13.9	15.2	7.41	7.60	7.2	20.5	44	68	6	2	50	29
283*	14.0	15.8	5.47	8.35	15.1	7.8	45	51	12	3	40	46
249	16.5	15.4	8.05	7.59	5.6	19.9	50	51	4	–	46	49
278	16.5	14.9	7.09	7.45	13.95	27.2	50	70	10	4	40	26

* Dogs 0260 and 0257 had 1% of basophils before duomycin; 0260 and 269 had 1% of monocytes after duomycin; and 283 had 3% of monocytes before duomycin.

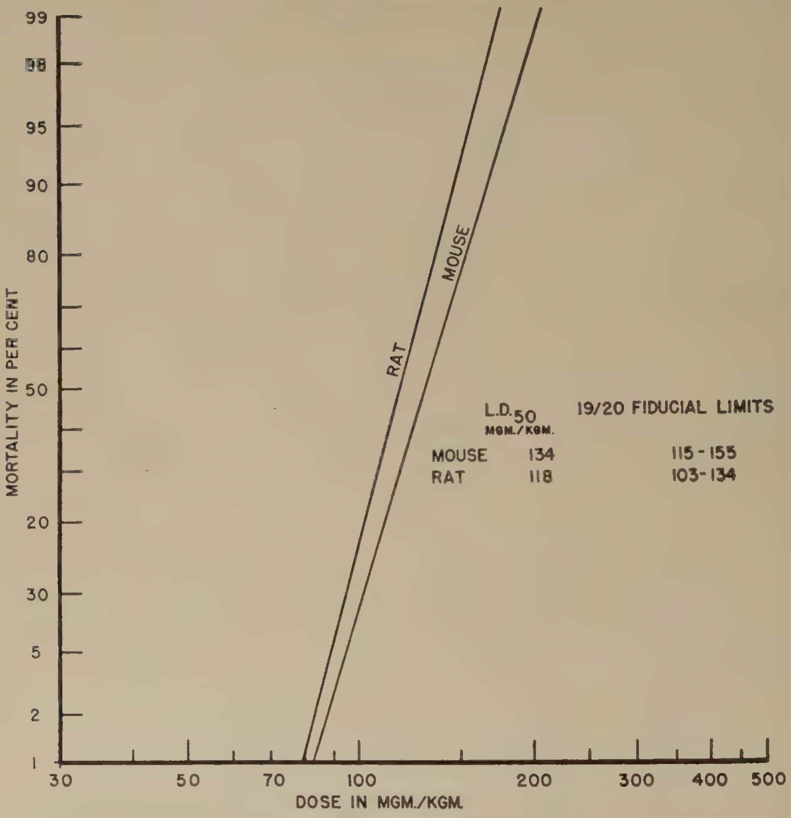

FIGURE 1. Acute intravenous toxicity of duomycin—pH 2.5. The experimental results were plotted on log probability paper and straight lines were fitted by eye. The 19/20 fiducial limits were estimated by a modification of the method of Litchfield and Fertig.[9] The modification allowed for the fact that the population was not homogeneous in all cases and consisted of correcting the estimated values by multiplying by: $\sqrt{(Chi)^2/n}$, as described by Wilcoxon & McCallan.[10] The range of weights in grams was: mice 14–25; rats 100–315. Injection rates in mgm/kgm/min. were: mice 250–500; rats 30–40.

carbonate buffer, pH 8.5, was given to two of these dogs. No serious symptoms were observed. Two other dogs were given intravenously, three 20 mg. per kg. doses, and orally two 50 mg. per kg. doses in 24 hours. One dog vomited an oral dose but it was repeated later and retained. No other unfavorable symptoms were observed.

Three cats easily tolerated 50 mg. per kg., pH 8.5, injected intravenously at a rate of 50 mg. per kg. per minute. One cat vomited but other unfavorable reactions were not observed. Three rabbits and three guinea pigs were given intravenously 50 mg. per kg., pH 8.5, at a rate of 10–15 mg. per kg. per minute. There were no objectionable symptoms.

Subcutaneous, intramuscular and intraperitoneal doses at pH 2.5, 7.0 and 8.5 are irritating, and this factor plus the low solubility at the pH of the tissues renders the validity of toxicity data thus obtained open to question. Mice and guinea pigs tolerated subcutaneous doses of 300 mg. per kg. (TABLE 1). Rats and mice tolerated 200 mg. per kg. intraperitoneally but this dose killed fifty per cent of the guinea pigs.

Tests for methemoglobin in mice, rats, guinea pigs, rabbits, cats and dogs were negative.

Subacute Toxicity

Multiple Intravenous Doses to Unanesthetized Dogs. Over a period of eight days, six dogs were each given 270 mg. per kg. intravenously. All injections were made into the saphenous veins. Toward the end of the period, there was a definite amount of swelling near the sites of the injection, probably due to small amounts of the compound which entered the tissues around the veins. The dogs were lame and it is believed that the physical discomfort caused a loss of appetite which led to a decrease in weight (TABLE 1-A). It is interesting to note that one month after the administration of the compound, the weights of the dogs were equivalent to, or in excess of their predosing level. Their condition was excellent and although no biopsies were made the injected veins appeared normal.

Electrocardiograms were made before the drug and on the fourth day of the injections. No changes were noted. The data recorded in TABLE 1-A show that there were no changes in the complete blood counts, clotting time, liver function or kidney function.

Multiple Intramuscular Injections. Five guinea pigs were given 40 mg. per kg., pH 8.5 two times per day for four consecutive days and again on the sixth day. A second group of five was given half as much on the same schedule. There were no changes in hemoglobin or the total number of red blood cells in either group. Mice treated similarly had nine per cent reduction in hemoglobin and in red cells. Differences between the two doses were not significant. One mouse in each group, and one guinea pig on the 40 mg. schedule died.

Two dogs, No. 541 and No. 547, six months of age, were given eight 20 mg. per kg. doses of duomycin, pH 8.5 during a five day period. Dog 541 showed no change in weight, hemoglobin or red blood cells. Dog 547 lost ten per cent of his weight, thirteen per cent of his hemoglobin and twenty-one per cent of his red blood cells. Neither dog showed a change in the bromsulphalein test for liver function.

In all of the animals tested, intramuscular doses were irritating and produced an edema and tenderness in the injected area.

Chronic Toxicity of Oral Doses

Mice. Two groups of mice were given oral doses of duomycin pH 2.5. One group received 40 mg. per kg. and the other 100 mg. per kg. daily. At the end of the fourth week, the dose of the latter group was increased to 100 mg. per kg. two times a day. The growth curves for a twelve week period were the same for the treated groups as for the control group

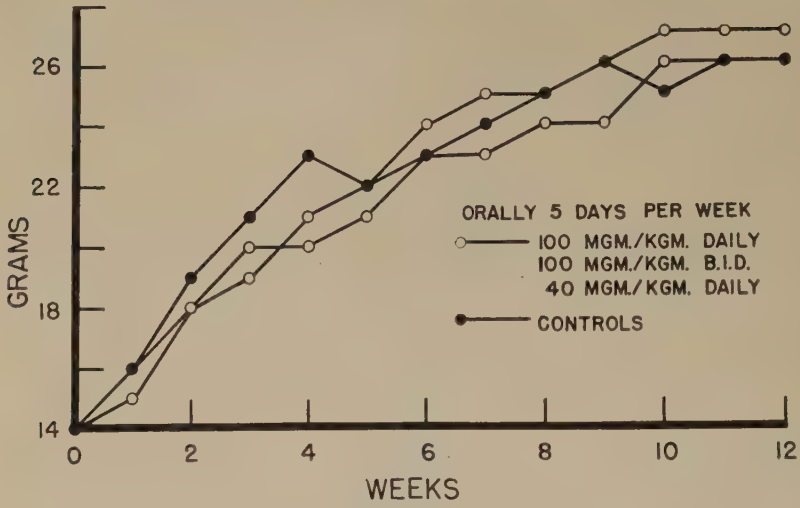

FIGURE 2. Growth curves on duomycin—male mice. After four weeks the dose of the group on 100 mgm/kgm was increased to 200 mgm/kgm.

Number of mice	*Start*	*End*
control	20	17
40 mgm/kgm	20	16
200 mgm/kgm	20	13

TABLE 2

EFFECTS OF MULTIPLE DOSES OF DUOMYCIN ON THE HEMATOLOGY OF RATS AND MICE

		Oral dose		*Hemoglobin*	*Total blood cell count*	
Animal	*Number counted*	*Per day* (mg./kg.)*	*Duration** (weeks)*	*During final week on duomycin (gms./100 cc.)*	*Red blood cells* *During final week on duomycin (millions per cu. mm.)*	*White blood cells* *During final week on duomycin (thousands per cu. mm.)*
Rat	8	200†	14	14.7	8.21	17.3
Rat	8	40	14	14.6	9.26	17.04
Rat	8	10	14	14.6	9.02	19.5
Rat	8	0	14	14.3	8.39	21.4
Mouse	10	200†	14	14.4	11.42	15.0
Mouse	10	40	14	12.9	10.04	13.5
Mouse	10	0	14	13.4	9.51	18.1

* The daily dose was divided into two equal portions and administered in the morning and afternoon
** Animals were dosed 5 days per week.
† For the first 17 doses the daily dose was 100 mg./kg.

(FIGURE 2). There were no unfavorable effects upon general appearance, the hemoglobin or the total blood cell counts (TABLE 2).

Rats. The data on rats are similar to those obtained on mice. The doses for the rat groups were identical with those used for mice except that an additional group was placed on 10 mg. per kg. per day (FIGURE 3). The

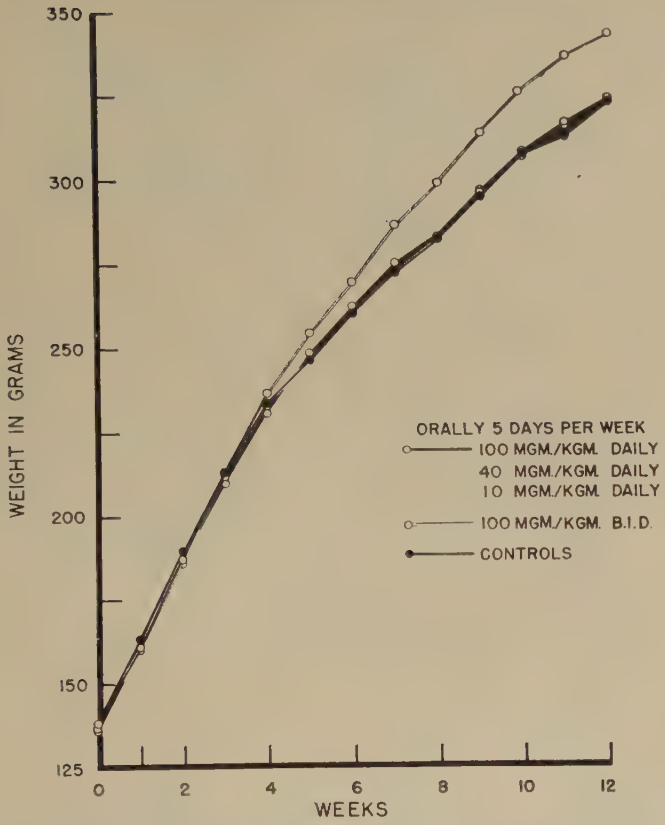

FIGURE 3. Growth curves on duomycin—male rats. After four weeks the dose of the group on 100 mgm/kgm was increased to 200 mgm/kgm.

Number of rats	*Start*	*End*
control	20	18
10 mgm/kgm	20	18
40 mgm/kgm	20	20
200 mgm/kgm	20	19

control group and the dosed groups grew at the same rate. The groups were indistinguishable and there was no effect upon the hemoglobin or blood cells (TABLE 2). At the end of the period, the blood pressure of these animals was tested by Mr. Ablondi with the method and apparatus recently developed in his laboratory.[7] There was no significant difference between the mean pressures of the groups. The blood sugars of the dosed and control groups were the same.

*Pathologist's Report.** Five mice from the control group, 10 from the 40 mg. per kg. group and 10 from the 100–200 mg. per kg. group were sacrificed. Similar numbers from the four groups of rats were also sacri-

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* The pathological examinations were made by E. Woll, M. D.

TABLE 3

THE EFFECTS OF MULTIPLE ORAL DOSES OF DUOMYCIN ON DOGS

	Oral dose		*Weight*		*Bromsulphalein test for liver function***				*Clotting time of blood†*	
					Before duomycin		*During final week on duomycin*			
					Time after injection of bromsulphalein					
Dog No.	*Per day* (mg./kg.)*	*Duration** (weeks)*	*Before duomycin (kg.)*	*Final day on duomycin (kg.)*	*5 min.* Retention *(per cent)*	*30 min.* Retention *(per cent)*	*5 min.* Retention *(per cent)*	*30 min.* Retention *(per cent)*	*Before duomycin (min.)*	*During final week on duomycin (min.)*
500	40	1								
	100	15	2.2	4.6	—	5	18	2	4.5	3.2
523	40	1								
	100	15	3.9	4.6	—	3	34	11	2.2	3.2
491	40	1								
	100	15	2.7	7.2	—	6	53	7	2.5	7.0
548	100	12	4.6	9.1	—	6	39	7	4.3	8.5
589	100	10	8.1	9.9	22	8	20	5	9.5	11.2
136	100	9	8.1	7.4	24	5	—	7	11.5	5.2
268	100	10	8.4	9.5	13	3	23	5	12.0	3.3
587	100	9	7.2	8.0	23	12	21	4	3.2	2.5
586	100	10	9.5	9.9	24	3	45	8	7.0	6.5
317	100	10	8.3	8.8	38	12	27	4	9.5	11.2

TABLE 3 *(continued)*

Dog No.	*Phenolsulfonphthalein test for kidney function*** *Before duomycin* *Two hour excretion (per cent)*	*During final week on duomycin* *(per cent)*	*Albuminuria*** *Esbach's test during final week on duomycin* *(per cent)*	*Blood pressure*†† *during final week on duomycin* *(mm./Hg)*
500	—	—	—	124
523	—	—	0	130
491	—	—	0	124
548	—	—	0	106
589	—	—	0	122
136	75	74	<0.025	110
268	85	66	0	144
587	76	68	0	114
586	80	81	0	124
317	85	84	0.025	116

* The daily dose was divided into two equal portions and administered in the morning and afternoon.

** Dogs were not dosed on Saturdays or Sundays. Tests for function were not made on Mondays.

† Capillary tube method.

†† From puncture of femoral artery.

TABLE 4

THE EFFECTS OF MULTIPLE ORAL DOSES OF DUOMYCIN ON THE HEMATOLOGY OF DOGS

			Total blood cell count				Differential count					
	Hemoglobin		Red blood cells		White blood cells		Neutrophils		Eosinophils		Lymphocytes	
Dog No.*	Before duomycin	During final week on duomycin	Before duomycin	During final week on duomycin	Before duomycin	During final week on duomycin	Before duomycin	During final week on duomycin	Before duomycin	During final week on duomycin	Before duomycin	During final week on duomycin
	(Gms./100 cc.)		(millions per cu.mm.)		(thousands per cu.mm.)		(per cent)		(per cent)		(per cent)	
500	11.4	13.9	5.30	7.00	19.3	9.7	—	69	—	0	—	31
523	11.6	17.4	4.85	8.85	23.8	8.2	—	75	—	2	—	23
491	11.4	13.9	5.90	7.15	17.2	12.2	—	46	—	4	—	50
548	10.2	13.1	5.10	7.50	—	10.0	—	47	—	0	—	53
589	9.6	10.5	5.15	5.90	9.3	12.6	71	52	1	14	28	34
136	12.2	13.6	5.45	6.70	21.6	21.8	53	71	30	24	17	5
268	17.2	14.5	8.50	6.20	14.9	10.4	68	46	4	12	28	42
587	15.6	12.1	7.50	6.10	10.3	13.8	49	74	5	6	46	20
586	14.5	16.8	7.65	7.95	11.0	14.8	84	72	2	14	14	14
317	14.9	15.6	7.50	6.50	8.1	6.8	49	50	12	18	39	32

* For doses see TABLE 3.

ficed. "No morphological changes attributable to duomycin were seen on gross or microscopic examination. In the mice and rats, there was some low-grade bronchitis. However, the incidence of spontaneous pulmonary pathology so common in this species was decidedly less in the treated groups."

Dogs. Ten dogs were given daily doses of 100 mg. per kg. orally in capsules for periods of 9 to 15 weeks. The essential data shown in TABLES 3 and 4 demonstrate that duomycin did not change the bromsulphalein test for liver function, the P.S.P. test for kidney function, the hematology or the clotting time. Seven of the nine dogs showed no albuminuria. Of the others, one had 0.025 per cent albumin and one less than 0.025 per cent. Through error, no test was made on dog No. 500. Tests for albuminuria were not made prior to dosing, hence it is not known if the traces found in the two dogs were present before duomycin. Blood pressures taken during the final weeks on duomycin were within the normal range. At the end of the experimental period these animals were in excellent condition.

*Pathologist's Report.** Dogs 587, 136, 523, 548, 589, and 491 were sacrificed and studied. Intestinal parasites were found in 589, 548, and 523. Heart worms were found in 136, nephrosclerosis in 587 and 136 and simple ovarian cysts in 587. The pathologist concluded "Duomycin administered orally in the above schedule did not produce any detectable gross or microscopic changes."

Chicks. Robbins' observation[8] that 2,4-dinitrophenol produced cataracts in the eyes of chicks prompted us to subject duomycin to similar test. Eighteen seven-day-old chicks were divided into groups of six and given the following compounds in the chick feed: Duomycin hydrochloride, 0.25 per cent; 2,4-dinitrophenol, 0.25 per cent. Plain chick feed was given to the controls. The chicks on 2,4-dinitrophenol developed cataracts overnight. The chicks on duomycin showed no lenticular changes after seven days of dosing. No chick in this group died. Two were lost from the control group. It is interesting to note that, although the average weight of the chicks in the two groups was the same at the start, at the end the birds on duomycin had an average weight of 99 grams as compared with 81 grams for the controls. Furthermore, the dosed chicks appeared to be more healthy than the controls and after 6 weeks this superiority was maintained. Apparently, the duomycin had eliminated some infection.

Tests for Pharmacological Activity

Blood Pressure. DOGS. The data in TABLE 5 and FIGURES 4, 5 and 6 show the effects on the blood pressure of small and large doses at pH 2.5 and 8.5 given to etherized and nembutalized dogs at different rates of injection. FIGURE 4 records an observation duplicated many times. Under the same conditions, the rapid injection of duomycin hydrochlo-

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* The pathological examinations were made by E. Woll, M. D.

TABLE 5

THE EFFECTS OF MULTIPLE INTRAVENOUS DOSES OF DUOMYCIN ON THE BLOOD PRESSURE OF ANESTHETIZED ANIMALS

		Dose					Blood pressure			
Animal	*Anesthetic*	*Number*	*(pH)*†	*(mg./kg.)*	*Time required for injection (seconds)*	*Interval between injections (min.)*	*Control (mm. Hg)*	*Fall†† Maximum (mm. Hg)*	*Rise‡ Maximum (mm. Hg)*	*Stabilized at (mm. Hg)*
Dog 611	Ether	1	8.5	10	25	—	120	6	4	124
		2, 3, 4, 5, 6, 7, 8, 9, 10	8.5	10	25	15	124–128	0	0	124–128
Dog 607	Ether	1	2.5	10	20	—	106	6	28	132
		2, 3	2.5	10	20	15	132	36, 30	0,18	132–150
		4, 5	8.5	10	20	22, 13	150, 152	3	2	152, 154
		6, 7	2.5	10	20	12, 29	154, 156	4, 8	2,0	156*
		8, 9	2.5	10	20	13, 18	160, 158	16	0	158, 156
Dog 608	Ether	1	2.5	10	20	—	160	18	34	194
		2, 3, 4, 5	2.5	10	20	15	194–190	10–22	0–6	194–186
		6, 7, 8	2.5	10	20	15	186–174	16–24	0	180–170
		9, 10, 11, 12	2.5	10	20	15	170–154	18–38	0	164–150
		13	2.5	10	20	15	150	38	0	146
		14	2.5	10	20	15	146	40	0	134
Cat 619	Ether	1, 2, 3, 4	8.5‡‡	10	8	10–15	104	0	0–2	104–106
		5	8.5‡‡	10	30	10	106	0	4	100
		6	8.5‡‡	50	60	15	100	0	22**	94
Cat 620	Ether	1	8.5‡‡	20	12	—	142	4	4**	140
		2, 3, 4	8.5‡‡	20	12	10	140–132	0	6–8**	138–122
		5, 6, 7	8.5‡‡	20	12	10	122–104	0	8–6**	122–98
		8, 9, 10, 11	8.5‡‡	20	12	10	98–88	0	6–12**	90–86
		12	8.5‡‡	20	12	12	86	0	14**	80
		13	8.5‡‡	50	10	10	80	12	24**	70
Cat 618	Ether	1	8.5‡‡	10	60	—	76	0	40**	112
		2, 3, 4, 5	8.5‡‡	10	10	10	112–110	6	4–8**	108–112

Animal	*Anesthetic*	*Number*	*(pH)*†	*(mg./kg.)*	*Time required for injection (seconds)*	*Interval between injections (min.)*	*Control (mm. Hg)*	*Fall*†† *Maximum (mm. Hg)*	*Rise*‡ *Maximum (mm. Hg)*	*Stabilized at (mm. Hg)*
Cat 661	Dial***	1, 2, 3, 4, 5	8.5	5	20	—	120–128	0	0–3	120–130
		6, 7, 8, 9, 10 11	8.5	5	20	5–10	130–120	0–3	0–3	130–120
Cat 662	Dial***	1, 2	8.5	5	20	5	100	0	0–2	100–102
		3, 4, 5, 6, 7	8.5	5	20	5–10	102–136	0	4–16	106–146
		8, 9, 10, 11	8.5	5	20	5–10	146	0	0	146
Cat 652	Dial***	1, 2	2.5	5	20	26	132, 136	10, 12	4	136, 140
		3, 4, 5, 6, 7	2.5	5	20	10	140–150	8–12	0	140–150
		8	2.5	5	20	10	150	4	0	150
Cat 654	Dial***	1	2.5	5	18	—	102	14	10	112
		2, 3, 4, 5, 6	2.5	5	18	10	112–108	12–20	0	106–104
Cat 657	Dial***	1	2.5	10	10	—	136	46	24**	138*
		2	2.5	10	30	10	152	22	16	168*
		3	2.5	10	60	10	144	6	18	162
		4	2.5	25	300	15	162	4	0	158*
		5	2.5	10	10	50	136	42	6	142
		6	2.5	10	30	10	142	32	8	150*
		7	2.5	10	60	10	146	28	6	152*
		8	2.5	25	300	15	158	6	0	158
Cat 655	Dial***	1, 2, 3	2.5	5	20	5–10	108–118	0	2–5	110–118
		4, 5, 6, 7, 8 9, 10, 11	2.5	5	20	5 or 10	118–142	10–28	2–8	120–150
Cat 653	Dial***	1, 2, 3, 4, 5	2.5	5	20	5 or 10	182–174	14–18	0	176–168
		6, 7	2.5	2.5	20	5 or 10	168–166	8	0	166

* When the stabilized level did not agree with the subsequent control value some other procedure had been interposed.
** Rise was followed by a gradual fall.
*** Dial–urethane. Each cc. contained: diallylbarbituric acid 0.1 gm.; urethane 0.4 gm.; monoethylurea 0.4 gm. The dose was 0.7 per cc. per kg., intraperitoneally.
† The concentration of duomycin was 1.0 per cent unless stated otherwise.
†† The duration of the fall usually approximated thirty seconds.
‡ The rise usually lasted two to ten minutes.
‡‡ The concentration of duomycin was 2.0 per cent.

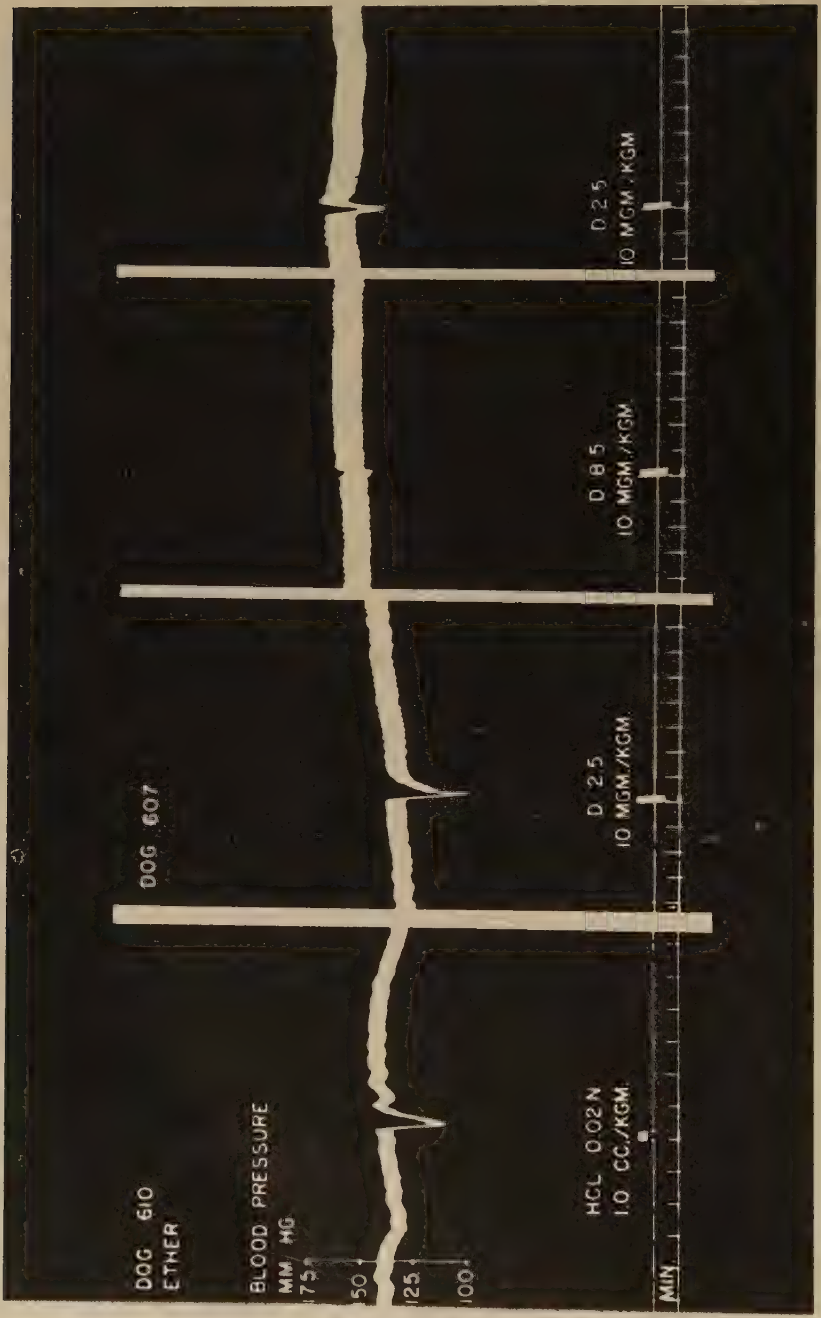


FIGURE 4. A comparison of the effects of an initial injection of duomycin hydrochloride pH 2.5 with an initial injection of hydrochloric acid equivalent in total acidity and volume. These are compared with the action of duomycin in a sodium carbonate buffer pH 8.5. All injections were intravenous.

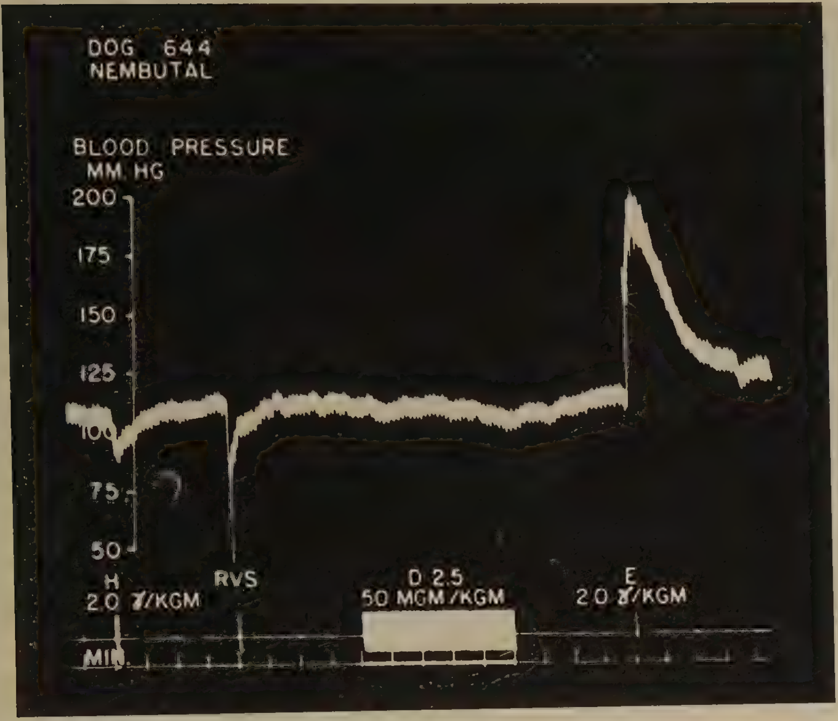


FIGURE 5. The effect of duomycin hydrochloride given intravenously at a rate of 10 mgm/kgm/min. The response to histamine diphosphate 2.0γ/kgm (H); stimulation of the right vagus (RVS); and to epinephrine 2.0γ/kgm (E) indicate that the vasomotor responses were normal.

ride at pH 2.5 produced a fall in blood pressure which is almost identical with that produced by an injection of hydrochloric acid equivalent in normality and total volume. In this figure, different dogs were used to permit comparison of initial doses. The results on dog 607 demonstrate also that the same dose and rate of injection of the alkaline duomycin affects the blood pressure less than the acid form.

The influence of the rate of injection may be seen by a comparison of the results shown in FIGURES 4 and 5. In dog 607 (FIGURE 4), the rate of injection was 30 mg. per kg. per minute and the blood pressure fell 31 mm. of mercury. In FIGURE 5, dog 644, the rate was 10 mg. per kg. per minute and no fall occurred even though the total dose was five times as great. In FIGURE 6, the rate of administration was 20 mg. per kg. per minute of the alkaline duomycin and the first injection produced a fall of 20 mm. of mercury, but the second injection of the same dose at the same rate produced no change in the blood pressure. The vasodepressor action of this rate appeared to be borderline.

The total dose tolerated in a relatively short period is high. Dog No. 611, TABLE 5, was given 100 mg. per kg., pH 8.5 in 140 minutes. After

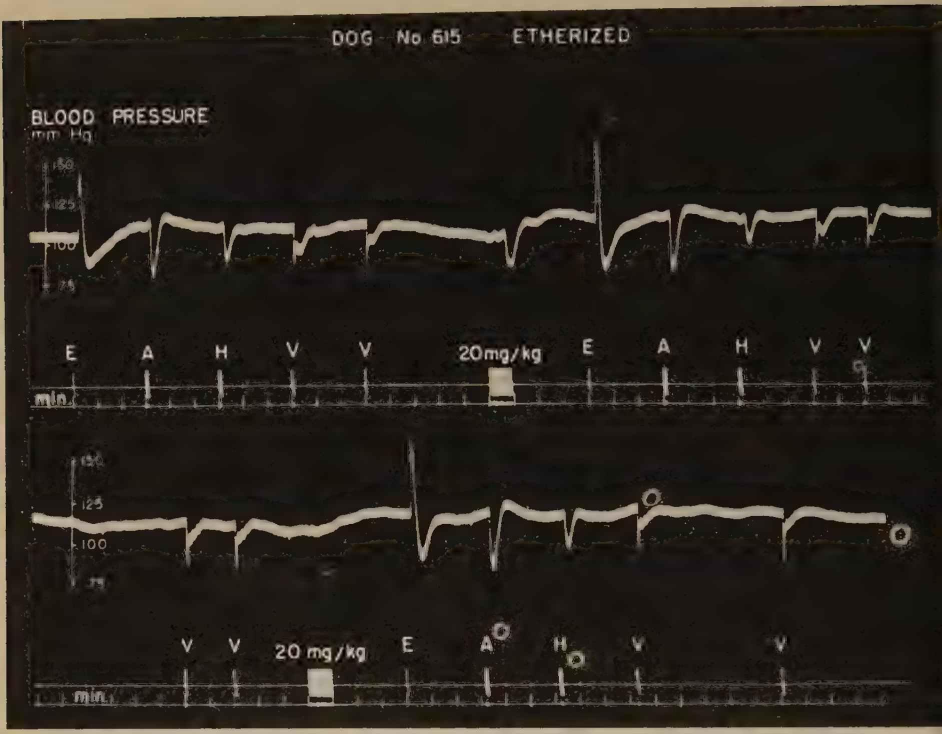


FIGURE 6. The effect on the blood pressure of the intravenous injection of duomycin pH 8.5 at the rate of 20 mgm/k
min. The vasodepressor action of this rate appears to be border line. Duomycin did not modify the vasomotor respon
of: epinephrine, 2.0γ/kgm (E); acetylcholine chloride, 0.4γ/kgm (A); histamine diphosphate, 2.0γ/kgm (H); stimulat
of the right vagus (V). See also TABLE 6.

the last injection, the blood pressure was 128 mm. of mercury, 8 mm. above the control value. Dog 608 was given 140 mg. per kg., pH 2.5, in 215 minutes. The blood pressure before duomycin was 160 mm. of mercury and after the last dose it was 134. In dog 607, the first injection of duomycin pH 2.5 raised the blood pressure from 106 to 132. After eight more injections of 10 mg. per kg. each during a period of 110 minutes, the pressure was 156 mm. of mercury. Dog No. 665 anesthetized with nembutal was injected intravenously with the alkaline duomycin pH 8.5 at a rate of 6.25 mg. per kg. per minute. During a period of forty minutes, a total dose of 250 mg. per kg. was given. The control blood pressure of 143 mm. of mercury remained essentially unchanged and at the end of the injection the pressure was 146.

In 4 unanesthetized dogs, the intravenous injection of 20 mg. per kg. in thirty seconds produced no important changes in the blood pressure (FIGURE 9). The dog with the greatest change was a poorly trained animal with an initial blood pressure of 140, a pressure just before the injection of

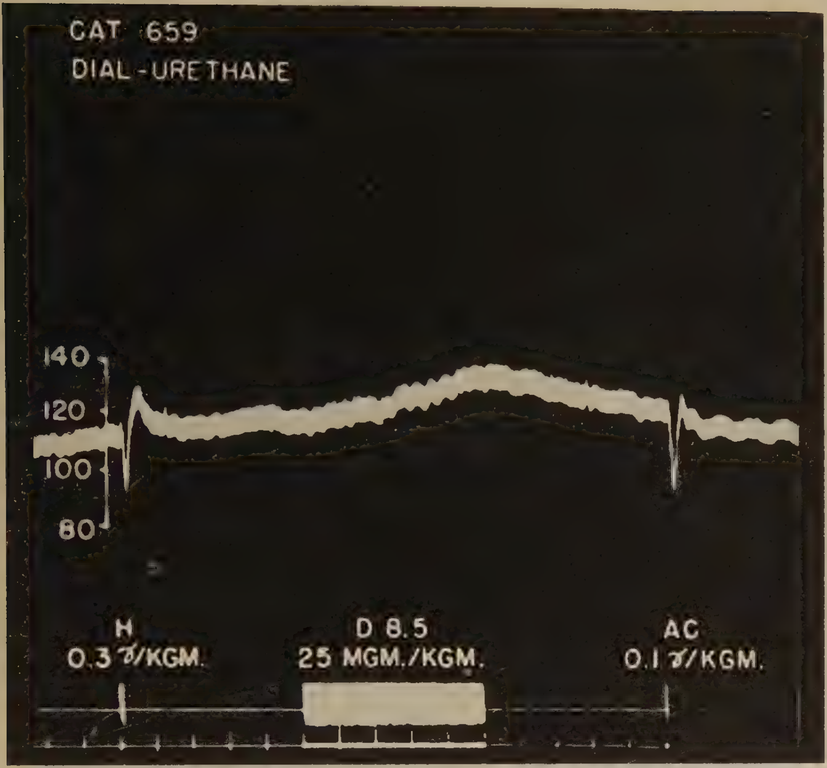


FIGURE 7. The effect on the blood pressure of duomycin pH 8.5 injected intravenously at the rate of 5 mgm/kgm/min. The responses to histamine base (H) and to acetylcholine chloride (AC) were normal.

128 and a final pressure of 118. The excitement incident to the preparation of the dog may account for the higher control value.

CATS. The blood pressure data on cats anesthetized with dial-urethane or with ether present a picture almost identical with that obtained on dogs (TABLE 5, FIGURES 7 and 8). Perhaps attention should be called to cat 646 (FIGURE 8). This animal was given 100 mg. per kg. of duomycin pH 2.5 at a rate of 12.8 mg. per kg. per minute. During the first few seconds of the injection the blood pressure fell from 170 to 120 and rose immediately to 165 mm. of mercury. This effect appears to be associated with the acidity of the solution, since a similar effect was observed after the injection of hydrochloric acid. After 35 to 40 mg. per kg. of duomycin had been injected, the pressure fell gradually to 85 mm. of mercury, but rose to 130 and stabilized as soon as injection had ceased.

Effects of Duomycin on Vasomotor Responses. In either the acid or alkaline form, duomycin does not modify the response to epinephrine, histamine, acetylcholine or faradic stimulation of the right vagus (FIGURES 6 and 8 and TABLE 6). The tests were made on dogs and cats anesthetized

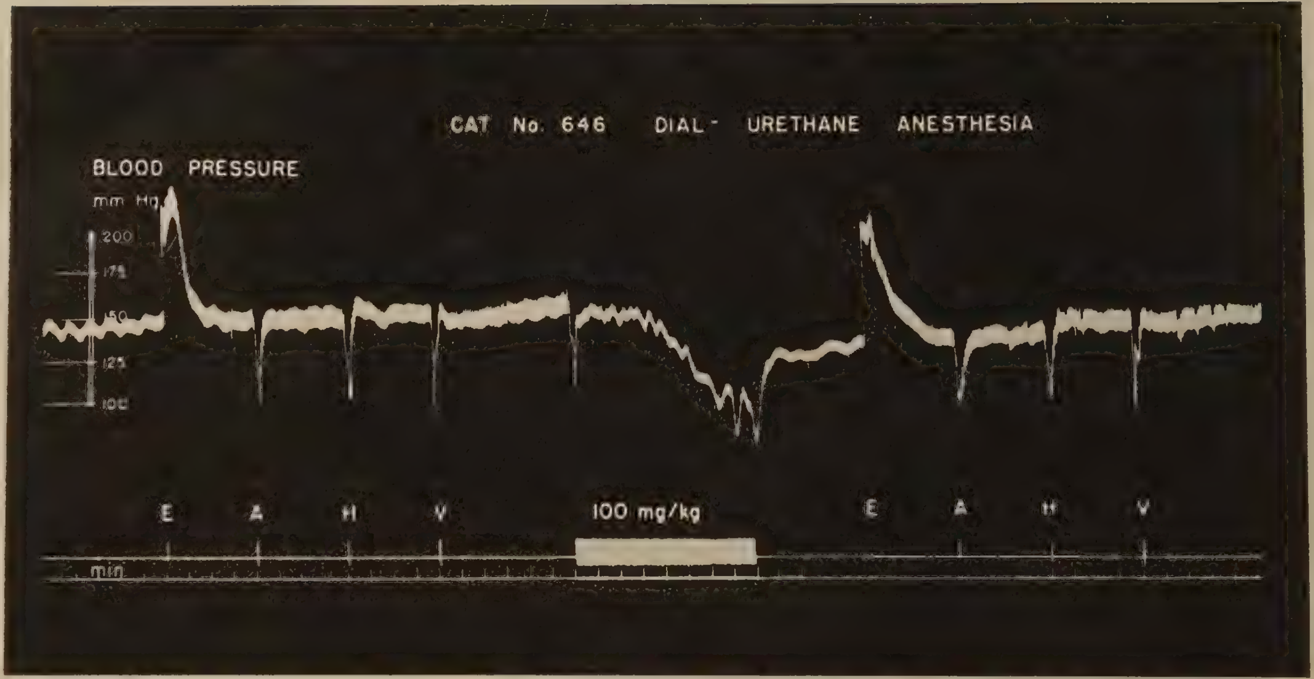


FIGURE 8. The effect on the blood pressure of the intravenous injection of a massive dose of duomycin pH 2.5 administered at the rate of 12.8 mgm/kgm/min. Duomycin did not modify the vasomotor responses of: epinephrine, 4.0γ/kgm (E); acetylcholine chloride, 0.1γ/kgm (A); histamine diphosphate, 0.8γ/kgm (H); stimulation of the right vagus (V). See also TABLE 6.

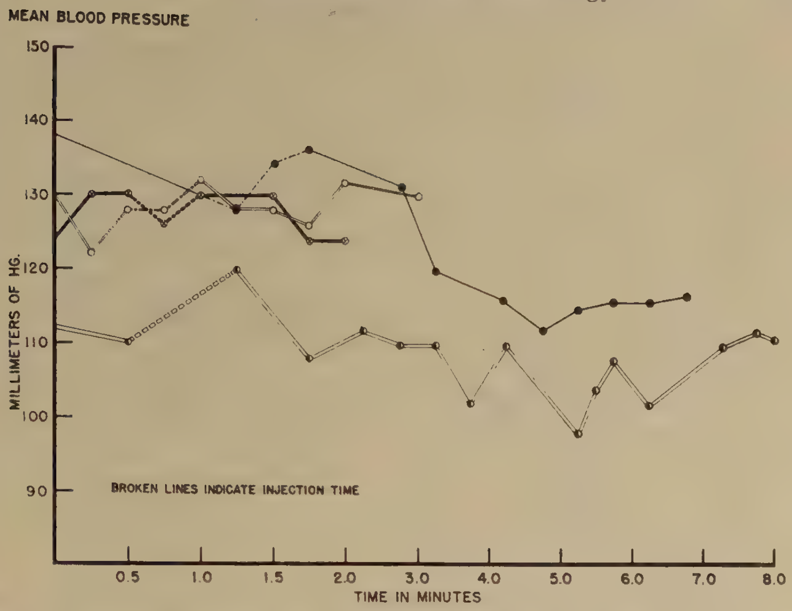


FIGURE 9. Duomycin pH 8.5, 20 mgm/kgm intravenously (unanesthetized dogs).

with ether, nembutal or dial and the doses of duomycin ranged from 20 to 100 mg. per kg.

Electrocardiograms. These studies made on nine unanesthetized and anesthetized dogs indicate that duomycin injected at a rate of 5 to 10 mg. per kg. per minute has essentially no effect upon the heart in doses up to 50 mg. per kg. The effect of doses between 50 and 100 mg. per kg. are minor and temporary and are probably more closely related to the quantity of acid or alkali than to the duomycin.

Respiration. The effects of duomycin upon respiration were studied in unanesthetized dogs, cats and rabbits, and in dogs and cats anesthetized with ether, nembutal or dial-urethane. Changes were unimportant and the effects observed could not be dissociated from those produced by an equivalent injection of acid or base. The massive doses recorded for anesthetized dogs and cats in TABLES 5 and 6 were never accompanied by respiratory depression.

Kidney. Duomycin hydrochloride has a mild diuretic action when assayed by the Lipschitz rat method.[4] When urea is assigned the value of 1.0, the activity of duomycin is 12.6. By the same assay, the diuretic potency of caffeine is 32 and that of theobromine 115. Tests for albuminuria were made on 176 rats used for the diuretic assay. Rats normally show a trace of albumin, less than 0.025 per cent, but this was not increased although the oral doses of duomycin hydrochloride were 50, 100, 200 and 400 mg. per kg. (TABLE 8).

In TABLE 7, the results from some intravenous doses in dogs and rabbits

TABLE 6

THE RESPONSE OF THE BLOOD PRESSURE TO EPINEPHRINE, ACETYLCHOLINE, HISTAMINE, AND FARADIC STIMULATION OF THE RIGHT VAGUS BEFORE AND AFTER INTRAVENOUS ADMINISTRATION OF DUOMYCIN

				Blood pressure					*Blood pressure*				
				Control		*Epinephrine-rise*			*Control*		*Acetylcholine-fall*		
		Dose of duomycin†		*Before Duomycin*	*After Duomycin*	*Before Duomycin*	*After Duomycin*	*Interval between duomycin and epinephrine*	*Before Duomycin*	*After Duomycin*	*Before Duomycin*	*After Duomycin*	*Interval between duomycin and acetylcholine*
Animal	*Anesthetic*	*(pH)*	*(mg./kg.)*	*(mm. Hg)*	*(mm. Hg)*	*(mm. Hg)*	*(mm. (Hg)*	*(min.)*	*(mm. Hg)*	*(mm. Hg)*	*(mm. Hg)*	*(mm. Hg)*	*(min.)*
Dog 614	Ether	8.5	20	150	148	44	42	3	156	146	36	42	6
Dog 614	Ether		20		140		42	3		150		40	6
Dog 615	Ether	8.5	20	102	112	38	44	3	108	114	28	30	6
Dog 615	Ether		20		116		42	3		116		32	6
Cat 626	Ether	8.5*	60**	122	102	22	12**	5	124	96	24	18**	10
Cat 618	Ether	8.5*	110^{++}	76	118	44	22^{++}	25	90	112	34	32^{++}	
Cat 620	Ether	8.5*	290^{+++}	142	70	16	22^{+++}	5	142	66	20	14^{+++}	
Cat 649	Dial***	8.5	50	160	134	34	48	5	162	142	20	14	10
Cat 659	Dial***	8.5	25						106	122	20	26	5
Cat 650	Dial***	8.5	50	142	132	36	42	3	136	130	38	30	6
Cat 650	Dial***		100		126		36	3		124		28	6
Cat 658	Dial***	8.5	25						120	104	22	22	5
Cat 658	Dial***		25^{++++}						110	112	26	24	5
Cat 658	Dial***		25^{+++++}						116	116	0	0	5
Dog 644	Nembutal, Sodium	2.5	50	100	112	80	80	4	110	126	8	8	8
Dog 644			100		124		80	30		120		12	34
Cat 647	Dial***	2.5	50	108	94	68	64	5	112	96	26	14	10
Cat 646	Dial***	2.5	50	170	148	54	62	10	162	150	30	46	14
Cat 646	Dial***		100		134		64	5		136		38	9

TABLE 6 (*continued*)

	Blood pressure					*Blood pressure*				
	Control		*Histamine-fall*			*Control*		*Vagal-fall*		
Animal	*Before* *Duomycin* (*mm. Hg*)	*After* *Duomycin* (*mm. Hg*)	*Before* *Duomycin* (*mm. Hg*)	*After* *Duomycin* (*mm. Hg*)	*Interval between duomycin and histamine* (*min.*)	*Before* *Duomycin* (*mm. Hg*)	*After* *Duomycin* (*mm. Hg*)	*Before* *Duomycin* (*mm. Hg*)	*After* *Duomycin* (*mm. Hg*)	*Interval between duomycin and stimulation of vagus* (*min.*)
Dog 614	150	144	20	28	9	142	146	34	96	12
Dog 614		148		26	9		142	34‡	34	12
Dog 615	108	110	18	12	9	110	112	38	26	12
Dog 615		112		18	9		118	30‡	26	12
Cat 626						122	96	16	10**	15
Cat 618										
Cat 620										
Cat 649	162	146	32	32	15	158	146	40	26	20
Cat 659	110	114	16	18	10					
Cat 650	134	134	36	36	9	138	136	56	56	12
Cat 650		124		34	9		124		48	12
Cat 658	116	118	14	22	10					
Cat 658	108	116	0	0	10					
Cat 658	116	120	0	0						
Dog 644	106	122	14	14	12	112	122	68	76	16
Dog 644		116		8	38		114		60	42
Cat 647	106	90	16	8	15	92	82	34	40	20
Cat 646	160	148	42	42	18	154	152	66	58	22
Cat 646		140		34	13		148		50	17

† A 1.0 per cent solution was used except in cats 618, 620, 626. The doses were not cumulative unless so stated.

* Concentration of solution—2.0 per cent.

** Three injections of 20 mg./kg of duomycin at intervals of 10 minutes. Epinephrine, acetylcholine, histamine and vagal stimulation followed the last injection of duomycin.

†† Five injections of 10 mg./kg. of duomycin; one 20 mg./kg., and one 40 mg./kg. at intervals of 10 minutes. Epinephrine, acetylcholine, histamine and vagal stimulation followed the 40 mg./kg. of duomycin.

††† Twelve injections of 20 mg./kg. of duomycin and one 50 mg./kg. at intervals of 10 minutes. Epinephrine, acetylcholine, histamine and vagal stimulation followed the 50 mg./kg. dose.

†††† After 2.0 mg./kg., intravenously, of Tagathen (an antihistaminic drug).

††††† After Tagathen and 0.1 mg./kg. atropine sulfate, intravenously.

‡ New control.

*** Dial–urethane. Each cc. contained: diallylbarbituric acid 0.1 gm.; urethane 0.4 gm.; monoethylurea 0.4 gm. The dose was 0.7 cc. per kg. intraperitoneally.

Table 7

Tests for Albuminuria in Dogs and Rabbits Dosed Intravenously with Duomycin

	Intravenous dose		Time of collection of urines in hours subsequent to dose												
	(mg./kg.)	(pH)	Control sample	0.5	1	2	3	4	5	24	30	48	72	96	102
Dog No.			Albuminuria in per cent												
545	100	2.5	Trace <0.025							Trace <0.025	0			0	Trace <0.025
543	100	2.5	Trace <0.025	0.1		0.15				0.1	0				
542	50	8.5	0		0.1			0.1	Trace <0.025					Trace <0.025	0
556	50	8.5	0		0.1			0	Trace <0.025					0	0
Rabbit 666	50*	8.5	0							Trace <0.025					
Rabbit 667	50*	8.5	0									0			

* This dose was divided into two equal portions one of which was administered in the morning, the other four hours later.

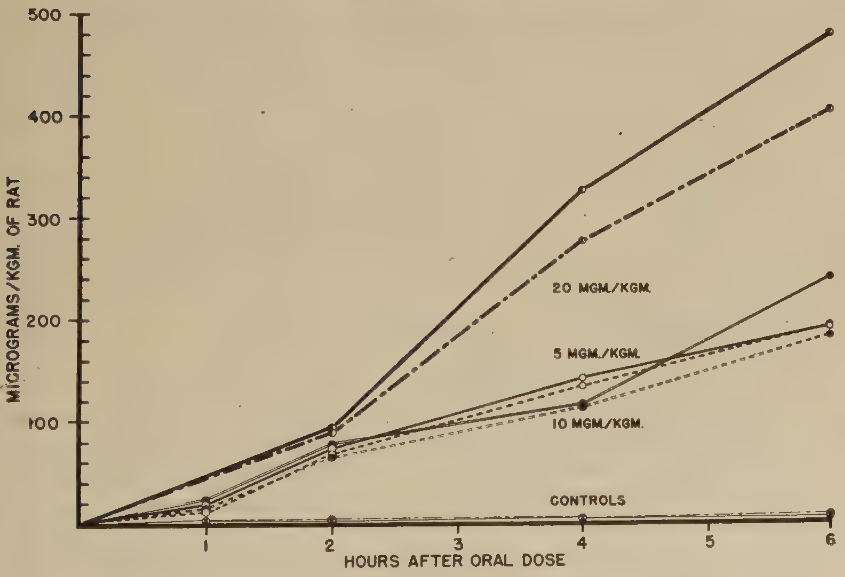
TABLE 8

TESTS FOR ALBUMINURIA IN RATS DOSED ORALLY WITH DUOMYCIN

Animal	*No. of groups*	*No. of rats (per group)*	*Dose of duomycin*		*Collection period (hours)*	*Albuminuria (per cent)*
			(mg./kg.)	*(pH)*		
Rats	12	8	0	—	0–5	Trace (<0.025)
Rats	2	8	50	2.5	0–5	Trace (<0.025)
Rats	4	8	100	2.5	0–5	Trace (<0.025)
Rats	3	8	200	2.5	0–5	Trace (<0.025)
Rats	1	8	400	2.5	0–5	Trace (<0.025)

have been recorded. Following doses of 50 to 100 mg. per kg. of duomycin, traces of albumin appeared temporarily in the urine of the dogs. Of the two rabbits given 50 mg. per kg., one showed a trace of albumin, less than 0.025, while the urine from the other was negative.

Urinary Excretion of Duomycin. One hour after the oral administration of duomycin hydrochloride to rats, the urine contained measurable quantities, even after doses as low as 5 mg. per kg. In FIGURE 10, it may be seen that the rate of excretion is relatively constant for at least six hours. Although the experiments were usually terminated in this period,



	TUBE DILUTION ASSAY				TURBIDIMETRIC ASSAY			
DOSE–MGM./KGM.	0	5	10	20	0	5	10	20
NO. OF EXPERIMENTS	6	5	4	3	4	4	2	2

FIGURE 10. Urinary excretion of duomycin (rats). Solid lines represent turbidimetric assay; the broken lines the tube dilution assay. Each experiment represents the urine from a group of four rats.

TABLE 9

DUOMYCIN—TESTS FOR IRRITATION IN THE RABBIT'S EYE

			Observations		
			Time in hours subsequent to final drop		
Rabbit No.	*Duomycin preparation*	*pH*	*1 hour*	*24 hours*	*48 hours*
1	0.5% in 0.9% NaCl†	2.9	mild irritation**	no irritation	no irritation
14*	0.5% in 0.9% NaCl†		mild irritation**	no irritation	no irritation
2	0.5% in 0.9% NaCl†		mild irritation**	no irritation	no irritation
15*	0.5% in 0.9% NaCl†		mild irritation**	no irritation	no irritation
3	0.5% in dist. H_2O	2.7	irritation	mild irritation	no irritation
16*	0.5% in dist. H_2O		mild irritation	mild irritation	no irritation
5	0.5% in dist. H_2O		mild irritation	mild irritation	no irritation
17*	0.5% in dist. H_2O		mild irritation	mild irritation	no irritation
4	0.9% NaCl	6.8	no irritation	no irritation	no irritation
6	0.9% NaCl		no irritation	no irritation	no irritation
11	0.9% NaCl		no irritation	no irritation	no irritation
7	1.0% in 0.9% NaCl	2.9	mild irritation	no irritation	no irritation
9	1.0% in 0.9% NaCl		mild irritation	mild irritation	no irritation
8	1.0% in dist. H_2O	2.5	mild irritation	mild irritation	—
18*	1.0% in dist. H_2O		irritation	mild irritation	—
10	1.0% in dist. H_2O		mild irritation	mild irritation	—
19*	1.0% in dist. H_2O		irritation	mild irritation	—
12	0.25% in 0.9% NaCl	2.9	mild irritation	no irritation	—
13	0.25% in dist. H_2O	2.8	no irritation	no irritation	—
20*	0.02N HCl	1.7	mild irritation	no irritation	—
21*	0.02N HCl		mild irritation	no irritation	—

Method used: One drop of the preparation was added to the left eye at five minute intervals until eight drops had been given.

* One drop of the preparation was added to the right eye at five minute intervals until three drops had been given.

** Mild irritation was manifested in temporary reddening of the conjunctiva.

† The borate complex appeared to be less irritating than the hydrochloride.

one experiment showed that significant amounts were excreted between the tenth and twelfth hour. The collection-vessels were kept in iced water to limit the decomposition of the drug. During the first six hours, 3.8 per cent of the administered dose of 5 mg. per kg. was excreted. At doses of 10 and 20 mg. per kg., two per cent of the dose was excreted in 6 hours.

After intravenous injections, the excretion in dogs is rapid for the first two hours, somewhat slower between the second and fourth hours, and low between the fourth and sixth hours (FIGURE 11). This figure shows a similar quantitative excretion after the injection of duomycin either pH 2.5 or 8.5.

Blood Sugar. Food was withdrawn from rats 16 hours before they were given 150 mg. per kg. of duomycin hydrochloride orally. Bloods were taken at 1.5, 3, and 5 hours after dosing and blood sugar determinations

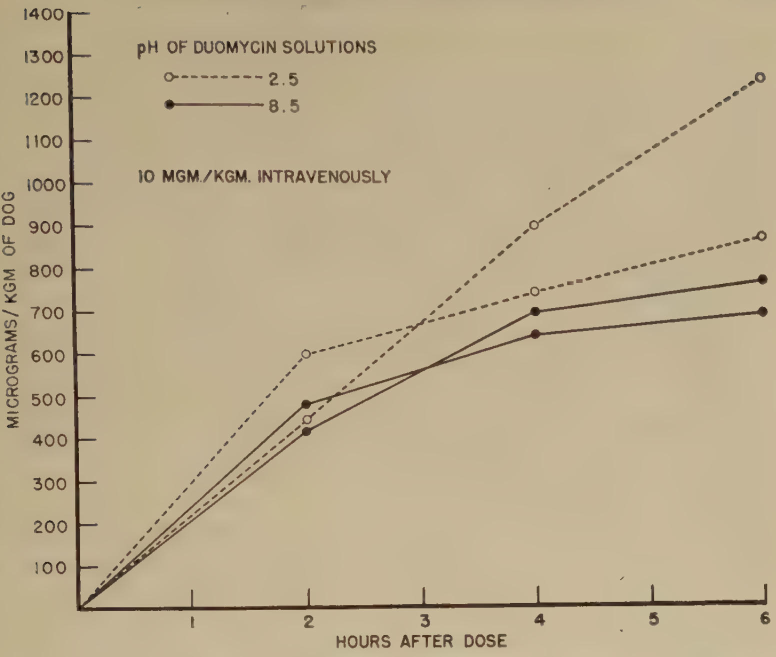


FIGURE 11. A comparison of the excretion of duomycin injected intravenously as the hydrochloride pH 2.5 and in a sodium carbonate buffer pH 8.5. Two dogs were injected with each solution.

were made on zinc hydroxide filtrate by the Shaffer-Somogyi method.[3] The average fasting blood sugar was 71 mg. per cent and there was no significant deviation from this value during the period of the test. Blood sugar determinations made on the dogs and rats used in the chronic dosing experiments were also normal.

Antihistaminic Action. Ten guinea pigs given intravenously 25 mg. per kg. 30 minutes before testing showed neither protection nor potentation of the effects of the standard histamine spray.

Action on Isolated Tissues. Duomycin had essentially no effect on the isolated gut from the guinea pig or rabbit. Tests for activity on the isolated uterus from these species were also negative.

Antipyretic Action. No evidence of antipyretic activity was observed in fevered rabbits and rats. FIGURE 8 is a composite of four experiments made on different days. The conclusion from each individual experiment was the same.

Irritation. Duomycin pH 2.5, 7, or 8.5 is irritating when injected intraperitoneally, intramuscularly, subcutaneously, or intracutaneously. In the rabbit eye, a 0.5 per cent solution of duomycin hydrochloride in 0.9 per cent saline is mildly irritating for a short time subsequent to the administration, but 24 hours later no evidence of irritation can be detected. The results are shown in TABLE 9. The mildness of the irritation

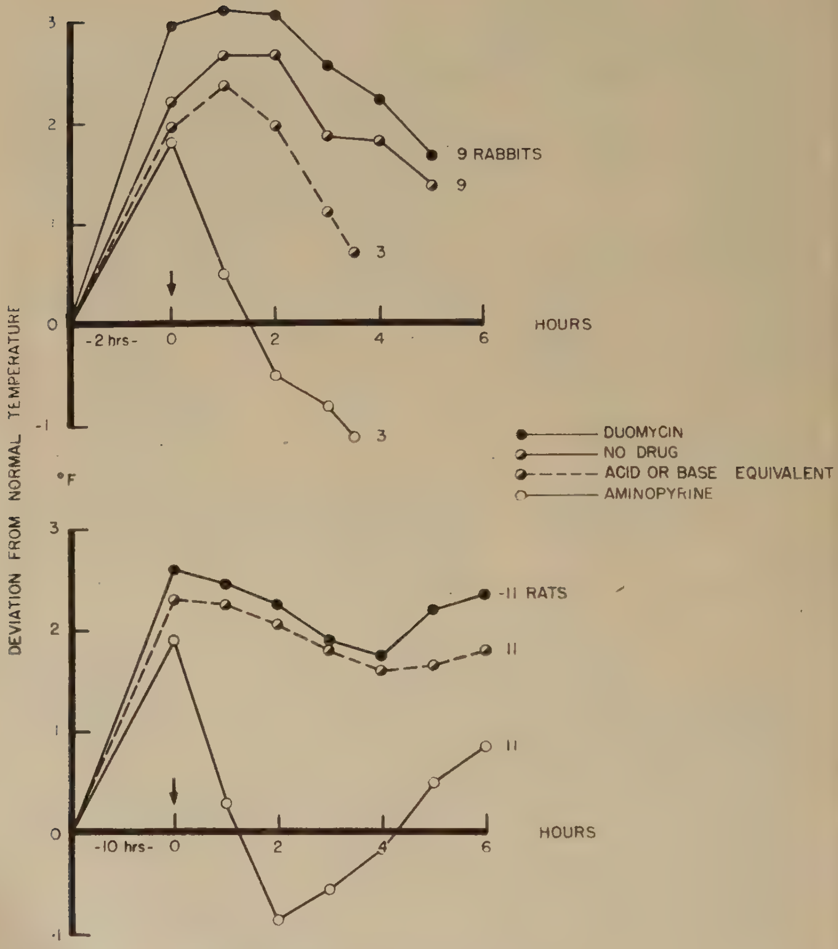


FIGURE 12. Antipyretic action of duomycin in rabbits and rats.

Fever was produced in rabbits by the intravenous injection of 0.25 cc. per kg. of combined typhoid vaccine. Two to two and one half hours were allowed for the development of the fever before the test-compounds were administered. At the time indicated by the arrows, duomycin pH 8.5 was injected intravenously in a dose of 25 mg. per kg; or aminopyrine, 50 mg. per kg., was given subcutaneously. Controls were given intravenously a solution of sodium carbonate-bicarbonate buffer equivalent in ratio, molarity and volume to the duomycin pH 8.5. Additional controls received no drug. The range of weight for the rabbits was 1.5 to 3.0 kg.

Rats were made febrile by the subcutaneous injection of 10 cc. per kg. of a 15 per cent suspension of dried yeast.[5] One group received orally 100 mg. per kg. of duomycin hydrochloride, a second group by the same route received 100 mg. per kg. of aminopyrine and the controls received a solution of hydrochloric acid equivalent in normality and total volume to the duomycin. The weights of the rats ranged from 200 to 300 grams.

apparently depends upon the fact that the pH and volume of the secretion prevent a prolonged contact.

Levels in Blood and Cerebrospinal Fluid. This study was confined to a convincing demonstration that duomycin passed the blood-brain barrier into the cerebrospinal fluid in therapeutically effective doses. No quantitative relationships between blood levels and cerebrospinal fluid levels were attempted. Dogs 468 and 200 were given 20 mg. per kg. intravenously at 8:40 A.M., 10:40 and 12:40 P.M. At 2:20 P.M., the arterial blood contained 40 gammas per cc. of serum, and at 2:30, the cerebrospinal fluid contained 0.8 gamma per cc. The results of tests on three other animals, dogs 273, 135 and 268, on the same schedule gave similar values. The cerebrospinal fluid from untreated dogs shows a titer less than 0.05 gamma per cc. Three undosed dogs were used.

Effect on Central Nervous System. In no animal was any effect on the central nervous system observed which could be attributed to duomycin as distinct from the acid or alkali administered with it. Doses of 50 mg. per kg. given intravenously at a rate of 10 mg. per kg. per minute to guinea pigs, rats, rabbits and dogs and at a rate of 150 mg. per kg. per minute to mice were tolerated with essentially no symptoms. Latent anticonvulsant or convulsant action was tested by determining the effect of 50 mg. per kg. intravenously in mice on the mortality produced by 100 mg. per kg. subcutaneously of metrazol. Of the 64 mice on metrazol alone, all convulsed and 44 per cent died. Of the 60 mice on metrazol, followed in 3 minutes by duomycin, all convulsed and 58 per cent died. This difference is not significant.

Summary and Conclusions

1. Duomycin, also known as aureomycin and as A-377, has a low toxicity and almost no side reactions.
2. Orally, mice tolerated 1500 mg. per kg. and rats 3000 mg. per kg.
3. The intravenous L.D.$_{50}$ for duomycin hydrochloride in mice was 134 mg. per kg., and for rats, 118. The alkaline form, pH 8.5, showed a toxicity of the same order.
4. Dogs, cats, rabbits, guinea pigs and mice tolerated without symptoms intravenous doses of 50 mg. per kg. pH 8.5 given at a rate of 10–20 mg. per kg. per minute. There was no evidence of methemoglobin formation.
5. Multiple intravenous doses of 20 mg. per kg. given to dogs two times per day for 6 days produced no unfavorable results except irritation of perivascular tissues at the site of injection.
6. Subcutaneous and intramuscular injections were irritating but 0.5 per cent solutions in 0.9 per cent saline produced only mild irritation in the conjunctival sacs of rabbits.
7. Mice, rats and dogs given 100 to 200 mg. per kg. per day orally for twelve weeks showed no evidence of chronic toxicity. The criteria were

growth, general appearance, hematology, clotting time, liver function, blood sugar, blood pressure and kidney function.

8. Duomycin pH 8.5 given to dogs intravenously at a rate of 10 mg. per kg. per minute produced essentially no changes in blood pressure or respiration. The hydrochloride, pH 2.5, was tolerated almost as well. There was one important difference. Doses of 30 to 40 mg. per kg. of the hydrochloride or an equivalent quantity of hydrochloric acid produced hemoglobinuria. This reaction was never observed with duomycin pH 8.5 even at 100 mg. per kg. Cats tolerated intravenous doses of the same order as those given to dogs.

9. Duomycin did not modify the vasomotor action of epinephrine, acetylcholine, or histamine; or the effect of vagal stimulation upon the heart.

10. Doses of 5 to 50 mg. per kg. of duomycin pH 8.5, given intravenously at the rate of 5 to 10 mg. per kg. per minute, did not modify appreciably the electrocardiograms of dogs.

11. Duomycin is a mild diuretic, about one third as active as caffeine. It does not produce albuminuria.

12. It has no effect upon blood sugar, isolated intestine or uterus and it does not potentiate or inhibit histamine.

13. It is not an antipyretic in rabbits or rats.

14. After oral doses, it appears in the urine in one hour and its excretion continues actively for 6 to 12 hours.

15. Therapeutically effective concentrations exist in the cerebrospinal fluid within 6 hours after an intravenous dose.

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BACTERIOLOGICAL STUDIES OF AUREOMYCIN

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PRELIMINARY studies of aureomycin have indicated that it is successful in the treatment of certain Rickettsial and viral diseases and, in addition, is effective against both the gram-positive and gram-negative types of organisms. In the early work on this drug, difficulty was encountered in the development of assay methods, particularly for the assay of serum concentrations in blood serum. It seemed important, therefore, to attempt to develop both cup-plate and serial dilution assays that could be depended on to evaluate aureomycin from the standpoint of both potency and its concentration in the blood following oral or parenteral use. Accordingly, sensitivities of a variety of organisms to the activity of aureomycin have been determined to establish the range of the bacterial spectrum for this drug and to identify organisms that would be useful in assay procedures.

The aureomycin* used in these investigations was relatively pure and was supplied as a sterile, amorphous hydrochloride. It was freely soluble in distilled water at a concentration of 2 per cent and produced a golden yellow solution having a pH of 4.5. All bacterial sensitivities were conducted in penicillin assay broth[1] utilizing the double serial dilution method, and a 1 per cent inoculum of a twenty-four hour broth culture of the desired organism. The tests were incubated at 37° C. for ninety-six hours, after which all negative tubes in the series were inoculated into fluid thioglycollate medium and incubated overnight to determine if any viable organisms remained. Table 1 presents these data, where it will be noted that gram-positive organisms are, in general, affected by much lower concentrations of aureomycin than are the gram-negative types. The most sensitive group studied appears to be the aerobic spore-bearing microorganisms. The variation between the bacteriostatic and the bactericidal endpoint was marked. When an organism is cultivated in the presence of aureomycin, inhibition of growth at the twenty-fourth hour may be brought about by very small concentrations, yet continued incubation results in growth in the tubes, and after incubation for ninety-six hours sub-culture in fluid thioglycollate broth reveals many surviving organisms in aureomycin concentrations hundreds of times greater than that inducing twenty-four hour inhibition of the same organism. Phenomena of this type could be due to the survival of resistant variants or

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*Supplied through the courtesy of Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

Table 1

	No. of strains	*Range of concentrations necessary to inhibit*		*Concentrations at which no viable cells remained*
		24 hours	*96 hours*	*96 hours*
Sal. typhosa	6	.78–1.56	25–50	25–50
Sal. typhi murium	1	1.56	50	50
Sal. pullorum	2	.39	12	25–50
Sal. derby	1	3.12	50	200
Sal. manhattan	1	3.12	50	200
Sal. oregon	1	3.12	50	200
			5	
E. coli	4	1.56–3.12		25–200
E. intermedia	1	6.25	50	50
St. hemolyticus	4	.19–3.12	6–25	25–50
St. fecalis	4	50	50	50–200
S. aureus	4	.09–25	3–50	50
S. citreus	1	.09	12	25
Kl. pneumoniae	6	.78–12.5	6–100	50–200
Pr. vulgaris	1	3.12	50	50
Ps. aeruginosa	2	50		
Sar. lutea	2	.09–.19	3–50	50
Sar. marcescens	3	6–50	100–200	100–200
Alkaligenes, sp.	1	.19	6	25
B. subtilis	4	.02–1.56	.78–12.5	1.5–50
B. megatherium	1	.02	3.12	3.12
Bacillus, sp.	3	.04–1.56	.78–6.25	1.5–25

to destruction of the antibacterial agent during the incubation period, or both.

Repeated transfers of representative organism were made in penicillin broth in order to determine whether a rapid increase in resistance to aureomycin could be induced. Increases of this type have been reported in the case of other antibiotics. Finland[2] reported that an increase in resistance of an organism *in vivo* during the administration of streptomycin often occurred within forty-eight hours, the increase in resistance being as much as 4,000-fold. One of the organisms employed in this study, *Salmonella typhosa*, showed an increase of only 4-fold in resistance to aureomycin after fourteen transfers, yet in an identical number of transfers through a medium containing streptomycin an increase of 226,000-fold was demonstrated.[3] Penicillin assay broth was employed as the medium. Serial dilutions by halves were made of the aureomycin and a 1 per cent suspension of the organisms added. The first tube showing growth was used as the culture for making up the suspension for each succeeding transfer. The results are given in TABLE 2, where it can be seen that even though some resistance does develop, with the exception of *Proteus vulgaris*, this increase in resistance could not possibly explain the variation between the bacteriostatic and bactericidal endpoints.

The stability of aureomycin in the presence of various culture media

TABLE 2

Microorganism	*Sensitivity* *Beginning* *μg/ml*	 *After 14 transfers* *μg/ml*	 *Increase*
B. subtilis	0.04	2.5	62
Sal. typhosa	1.2	5.0	4.1
E. coli	0.6	10	16.6
Kl. pneumoniae	2.5	80	32
Pr. vulgaris	1.2	640	533
Sar. lutea	.15	.3	2
S. aureus	.08	.08	0
St. hemolyticus	.15	10	66.6

and culture media ingredients was then studied to determine if certain compounds were responsible for a deterioration of the antibiotic. The concentration of aureomycin employed was 20 μg/ml and the deterioration, after incubation at 37° C., was measured by means of a *Bacillus subtilis* cup-plate technique to be described. Results are shown in TABLE 3. It thus appears that at 37° C. aureomycin is unstable even in water, and this instability is markedly increased in the presence of a variety of compounds and media. Penicillin assay broth, Thiol medium, experimental (Difco), various peptones, beef extract, and phosphate buffer at pH 7.0 resulted in almost complete destruction after twenty-four hours' incubation. Fluid thioglycollate medium, on the other hand, resulted in a destruction only slightly greater than that obtained with distilled water. Certain constituents of the fluid thioglycollate medium, when used in the concentration at which they appear in the finished medium, gave very marked loss of potency. Combinations of the various ingredi-

TABLE 3

Material tested	*Concentration* *%*	*% Loss in potency* *5 hours*	 *24 hours*
Penassay broth	—	43	96
Beef extract	0.15	35	90
Peptone	.5	24	76
Phos. buffer pH 7.0	.5	56	89
Fl. thioglycollate	whole medium	32	60
Casitone	.15	25	80
Yeast extract	.5	40	80
Dextrose	.5	8	46
Sodium chloride	.25	25	82
L-cystine	.1	20	51
Na thioglycollate	.05	20	50
Trypticase	1.5	20	55
Thiol medium (Difco)	3.0	25	98
N-Z case peptone	1.5	37	90
Water, distilled	—	15	46

ents to determine which were responsible for the apparent stabilization afforded by the complete medium are under investigation.

Various organisms were used in an attempt to develop a cup-plate assay method for aureomycin. One of these, *B. subtilis* 219, which is the organism used routinely in the assay of streptomycin,[1] was found to be the most satisfactory. The method is described below.

Prepare agar plates from penicillin assay agar[1] exactly as for the assay of penicillin but use *B. subtilis* PCI-219 for inoculating the seed layer. Place six standard penicillin assay cylinders on the seeded surface and fill the cups alternately with a dilution of unknown and with a reference standard made to contain 1.0 μg/ml. All unknowns and standard are dissolved or diluted with citrate buffer pH 6.3. Incubate the plates at 37° C. for eighteen hours and measure the zones of inhibition. The potency of the unknown solution is calculated using a standard curve. A typical assay for developing a standard curve for aureomycin is given in TABLE 4. All final dilutions are made in citrate buffer pH 6.3, since the use of a phosphate buffer not only rapidly inactivates aureomycin but results in "fuzzy" zones which are difficult to read.

TABLE 4

Concentration of aureomycin μg/ml.	*Zone of inhibition mm.*
0.1	14.5
0.4	19.3
0.6	20.7
0.8	21.7
1.0	22.7
1.2	23.3
1.5	24.1

The cup-plate assay method described was not applicable to the determination of aureomycin in blood serum because of poor zone formation and inconsistency in zone size. Serial dilution methods were then investigated using *B. subtilis* PCI-219 in a variety of media. The organism was found to be more sensitive to the action of aureomycin in fluid thioglycollate medium. This medium was also the one which resulted in the least destruction when aureomycin was incubated in its presence. The following test was devised.

Place 0.5 ml amounts of fluid thioglycollate medium in Wassermann tubes and serially dilute, by halves, the serum under test for the desired number of tubes. Place 0.5 ml of the serum in an empty Wassermann tube and place it first in the series. Prepare a standard for comparison by dissolving aureomycin in normal serum to give a concentration of 10μg/ml and prepare a duplicate fifteen tube series exactly as above. A one μg. standard can be used if it is made up fresh, kept under refrigeration, and used in the test within a few hours. Maintain the *B. subtilis* in penassay broth (Difco). Add 0.5 ml of a 1:100,000 twenty-four hour broth culture of the *B. subtilis* to each tube and incubate overnight at 25° C. The last tube showing no growth is considered the endpoint, and the concentration in the unknown is determined by comparing with the standard aureomycin series. Approximately 0.01 μg/ml of aureomycin is demonstrable with this procedure. A representative determination is given in TABLE 5.

TABLE 5

Tube No.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*Concentration*
Standard	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	
Standard	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	
Serum	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	10.0 µg/ml
Serum	−	−	+	+	+	+	+									0.02 µg/ml
Serum	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	5.0 µg/ml

(−) No Growth (+) Growth

Animal protection tests were conducted using six microorganisms. All injections were made intraperitoneally, utilizing 0.5 ml of the culture, followed immediately by a single appropriate dose of aureomycin in 0.5 ml of distilled water. The results are given in TABLE 6.

TABLE 6

Microorganism	*Aureomycin µg.*	*No. of mice*	*Per cent deaths*
S. typhosa	500	20	0
	250	20	10
	125	20	25
	50	40	35
	40	40	85
	25	40	100
	10	60	100
	0	60	99
S. typhi murium	500	20	90
	250	20	90
	125	20	95
	0	20	95
E. coli	500	20	0
	250	20	10
	125	20	10
	10	20	10
	0	20	70
Kl. pneumoniae	50	20	100
	25	40	100
	0	20	100
Pr. vulgaris	50	20	15
	25	20	20
	10	20	70
	0	20	100
St. hemolyticus	50	20	35
	25	20	50
	10	20	60
	0	20	100

Aureomycin behaved somewhat differently than penicillin or streptomycin when the latter drugs were employed in an identical experiment. When penicillin or streptomycin was used, the animals surviving were normal by the forty-eighth hour, while many of the animals treated with aureomycin died four or more days after infection. When the effectiveness of aureomycin against experimental *S. typhosa* infections is compared with the effectiveness of streptomycin against the same infection on a weight basis, it is about 1/100th as active.

An LD_{50} was determined, using white mice. All injections were by way of one of the tail veins, using a five second injection period. The results appear in TABLE 7.

TABLE 7

Dose			
Per 20 gm.	*Per kilo*	*Spasms*	*Deaths*
1.50 mg.	75.0 mg.	4/10	0/10
1.75	87.5	8/10	4/10
2.00	100.0	10/10	5/10
2.25	112.5	10/10	7/10
2.5	125.0	10/10	7/10
2.75	137.5	10/10	10/10
3.0	150.0	10/10	10/10

LD_{50} = 102/5 mg./kg. ± 0.32 mg.

The sample employed was found to be free from pyrogenic substances. When 20 mg./kg. of rabbit was injected the average temperature rise observed was 0.3° C. This dose, far in excess of the 1.0–2.0 mg./kg. of penicillin and 10.0 mg./kg. of streptomycin given in routine pyrogen testing, was well tolerated.

The effect of aureomycin on the blood pressure was studied in anesthetized cats. Phenobarbital was used as an anesthetic and the arterial blood pressure was determined by directly cannulating the carotid artery. When 10 mg./kg. was administered, a slight but transient fall in blood pressure occurred. At a dose of 20 mg./kg. the fall in blood pressure was more marked but a rapid recovery occurred with no evidence of respiratory embarrassment in any of the animals. When the dose was increased to 40 mg./kg., precipitous falls in blood pressure occurred, accompanied by cardiac arrest. The respiration became very shallow but continued for some time. In one animal, following a 40 mg./kg. dose which induced an 80 mm. drop in pressure, cardiac arrest occurred, but a return to normal was obtained following manipulation of the heart. A second dose of this size, however, resulted in a precipitous fall in the blood pressure of 100 mm., after which the animal could not be revived.

When aureomycin was combined with penicillin in the treatment of experimental *S. typhosa* infections in white mice, a synergistic effect was observed ranging from 5–40 per cent of that expected from the additive effects of these two drugs. When aureomycin was combined with streptomycin, however, in similar experiments, the results were not consistent.

Discussion

It is apparent from these studies that the bacterial spectrum of aureomycin is somewhat similar to that of streptomycin, inasmuch as it has an inhibitive action against both gram-positive and gram-negative organisms. Its greatest activity appears to be against the gram-positive spore-bearing organisms. In general, it may be said that its activity is not as great as that of streptomycin, but its acute toxicity appears to be of approximately the same order of magnitude. On the basis of animal experimentation, the drug is well tolerated, although it cannot be utilized in concentrations on a weight basis as great as those that may be utilized with penicillin. This investigation indicates that aureomycin in solution is an unstable antibiotic and loses potency rapidly in broth, water and serum. Various media ingredients cause marked destruction. Such substances as peptone, beef extract, and even phosphate buffer at pH 6.3 materially reduce the activity of the drug in a relatively short period of time. It is apparent that the use of a thioglycollate medium tends to preserve the potency of aureomycin to a greater degree than the other media utilized. This is of importance in the bio-assay procedure for determining serum blood concentrations of this antibiotic. The assay procedure originally utilized in these studies was unsatisfactory, since the medium as a whole or the media ingredients caused rapid destruction of the drug during the testing procedure. This destruction has been considerably overcome by the assay procedure described using thioglycollate medium and incubation at 25° C. Under these conditions, it is possible to determine concentrations of aureomycin of approximately 0.01 μg. per ml. in serum. No outstanding usefulness for this antibiotic has been demonstrated by these animal investigations against the bacteria studied, although a definite synergistic effect has been demonstrated against certain strains of organisms with penicillin. The greatest promise for the drug thus lies in the treatment of certain viral and Rickettsial diseases.

Summary and Conclusions

1. The bacterial spectrum of aureomycin has been determined using a variety of gram-positive and gram-negative bacteria.

2. Both a serial dilution method of assay and a cup-plate assay have been developed.

3. In solution, aureomycin appears to be quite unstable and is affected by a variety of substances.

4. In the treatment of bacterial infections in mice, aureomycin is not as effective as either streptomycin or penicillin.

5. Aureomycin is a relatively non-toxic drug and is well tolerated by the animals used in these studies.

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THE DETERMINATION OF AUREOMYCIN IN SERUM AND OTHER BODY FLUIDS

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THE potencies of antibiotic substances have been measured by several different methods. The most simple and perhaps the most widely used technique is that involving the serial dilution of the sample with agar[1] or with broth[2, 3] and the noting of the last dilution at which complete inhibition of the test organism occurs. Turbidimetric assays[4, 5] are merely refinements of the dilution assays wherein the antibiotic substance is diluted in such a manner as to produce an end-point which will fall intermediate between complete inhibition and no inhibition. Agar diffusion methods[6] similarly involve applying appropriate dilutions of the antibiotic in question to an inoculated agar surface and, after suitable incubation periods, measuring the zones of inhibition. With any assay method, it is important that a control solution be assayed in parallel with the "unknown" samples in order to have a point for comparison.

When the above methods were used in an attempt to assay aureomycin solutions, it was observed that the agar diffusion techniques as we applied them failed to produce sharp zones of inhibition. Also, the slope of the inhibition response curve was so low as to indicate a rather low order of precision. In making a survey of the various organisms readily available to us in the laboratory, we noted differences in the sensitivity of these organisms to aureomycin when tested by dilution methods. Since in selecting a test organism we must consider factors other than sensitivity alone, we eliminated those organisms which might be regarded as pathogenic. We did this not only for reasons of safety of laboratory personnel, but since we were primarily interested in assaying blood specimens we wished to eliminate the possibility of interference from specific bacteriostatic substances present in blood. In addition to the authentically identified cultures from our collection, we tested a number of laboratory isolates. One of these, Bacillus No. 5, seemed to meet our specification. It is a gram-positive spore-forming bacillus and appears to be a strain of *Bacillus cereus* variety *mycoides.*[7]

The method finally adopted for the assay of serum samples is similar to the methods originally proposed by the Food and Drug Administration for the assay of penicillin[8] and streptomycin[9] in blood. One-half ml. of a 0.2 μg./ml. solution of an aureomycin reference solution is placed in a ½ x 4½ inch Wassermann tube. A second ½ ml. portion of the same solution is diluted serially two-fold through an additional 5 tubes with sterile nutrient broth.[10] Samples submitted for assay are similarly diluted for as many tubes as may be required to give an end-point. If the approximate potency of the sample is known, it may be diluted with broth to contain approximately 0.2 μg./ml. after which it is serially diluted. When all of the samples and the standard have been placed in tubes, 1½ ml. of a 1:100 broth dilution of an overnight broth culture of the test organism is added with an automatic pipette and the tubes are incubated at 37° in a water bath. After 4 hours, the tubes are examined visually and the growth is recorded as either negative or positive. The end-point is usually quite easily distinguishable and even faint growth is recorded as positive. Quite consistently, we have had an end-point in the third tube of the standard series; thus, the smallest amount of aureomycin we are able to detect is 0.05 μg./ml. of sample. Actually, the aureomycin concentration in the final clear tube is one-quarter as much or 0.0125 μg./ml., since ½ ml. amounts have been made up to a volume of 2 ml. On several occasions, we have read the tubes at the 4-hour interval and then have incubated the tubes an additional 16 hours. Generally, the sensitivity of the assay is lowered to one-half or to one-quarter of that found at the 4-hour reading, but the actual potencies found in the samples do not change since the shift is parallel in the sample tubes and in the standard tubes. In the case of non-sterile urine specimens, when the assay tubes are incubated for a total of 20 hours, growth of the contaminating organisms may partially obscure the end-point. Even with grossly contaminated samples, however, we have usually been able to read end-points at 20 hours by observing the point at which the characteristic pellicle of Bacillus No. 5 occurs in each series. Because of the massive inoculum used initially, we have not had any interference from contaminated samples when they were read at the 4-hour interval. A total of 38 normal human urine specimens and 23 normal human serum specimens were examined with no indication of interference from inhibitory materials. Similarly, when we have fortified serum with aureomycin we have had no indication of interference from growth stimulation under the conditions of the assay.

Some of the early assay of blood and urine samples were carried out with *Bacillus subtilis* A.T.C.C. 6633. In these assays, the technique was identical with that used at present, but the tests were not as sensitive nor was it possible to read the tubes at the 4 hour time interval.

In addition to the dilution assay outlined above, we have utilized a turbidimetric assay for the determination of aureomycin in urine. As in the case of the dilution assay, we have modified existing penicillin techniques.[5] The urine samples are diluted with 0.1 M monobasic potassium phosphate to contain approximately 0.1 μg. of aureomycin per milliliter. Aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 ml. of this solution are added to unplugged 18 x 150 mm. test tubes and the volume of each is adjusted to 1.0 ml. with the same 0.1 M phosphate using a Cannon automatic dispenser. An aureomycin reference solution is included in each assay and it is set up at ten levels in a range from 0.01 μg. to 0.1 μg. per tube. The test organism, *Staphylococcus aureus* F.D.A. 209P, is carried out on nutrient agar slants. On the day of the assay, growth from a fresh slant is suspended in 150 ml. of nutrient broth,[10] and the inoculated flask is maintained at 37° for exactly 1½ hours. A bottle containing the calculated amount of broth for the entire assay is inoculated with this culture at the rate of 35 ml. per liter. Nine-ml. amounts of the inoculated medium are dispensed in each assay tube with an automatic pipetting machine. After incubating exactly 3 hours at 37°, ½ ml. of formalin 1 : 3 is added to each tube and the turbidities are read on a photoelectric colorimeter.

Twelve normal human urine samples were pooled and aliquots of the pooled material were fortified with different amounts of aureomycin. When tested by both of our assay methods, the recoveries were satisfactory. Similarly, we have had good agreement between the parallel assays performed on various animal urine samples submitted to us by the pharmacology group.

Using the turbidimetric assay we have made some aureomycin stability determinations. The antibiotic is most stable near pH 2. An unbuffered aureomycin hydrochloride solution at pH 2.9 has been maintained at cold room temperature ± 4° C. for 23 days with no measurable loss of activity. Temperature is an important consideration. Whereas aureomycin solutions from pH 1 through pH 10 have been held at ± 4° for approximately 18 hours with no loss in activity, at pH 7.0, more than half of the activity is lost after overnight incubation at 37°.

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IN VITRO STUDIES WITH AUREOMYCIN*

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INVESTIGATIONS on aureomycin have been in progress in this laboratory since November 1947. The *in vitro* activity of this new antibiotic against various bacteria has been compared with the activity of penicillin, polymyxin, and streptomycin, and the results are being reported elsewhere.

To recapitulate these results briefly, it was found that, in the test tube, aureomycin is less effective than polymyxin and penicillin against the gram negative bacilli and the gram positive cocci, respectively. The only exception noted was in the case of *Streptococcus faecalis*, six strains of which were found to be more susceptible to aureomycin than penicillin. For gram negative bacilli including *E. typhosa*, *E. coli*, *K. pneumoniae*, *A. aerogenes*, and *H. influenzae* β, aureomycin was found to be inhibitory at levels of from 2.5 to 5.0 micrograms per cc. of medium. Both *B. proteus* and *B. pyocyaneus* were found to be relatively resistant to aureomycin as neither one was inhibited by concentrations of 20 micrograms per cc. All of the gram positive cocci tested, including several strains each of staphylococci, pneumococci and hemolytic streptococci, were inhibited by less than one microgram of aureomycin per cc.

In a few instances, comparison with streptomycin was also made. Against *E. coli* and *K. pneumoniae*, aureomycin was not as effective as streptomycin. One of the test strains of staphylococci was, however, more susceptible to the new antibiotic than streptomycin. Both agents were equally effective against one strain of *H. influenzae* β.

In the course of the above studies on aureomycin, evidence began to appear suggesting that this antibiotic is bacteriostatic rather than bactericidal in action and that it deteriorates rather rapidly in the usual test media. The following investigations were undertaken in an attempt to throw more light on these phenomena.

Methods

1. Growth curves were obtained by making bacterial counts at various intervals after inoculation. The test organism used in this experiment was a beta hemolytic streptococcus, strain C203. Penicillin was chosen as the agent of comparison. Ten cc. volumes of both agents were inoculated

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* These investigations were supported by grants from Abbott Laboratories; Eli Lilly and Company; Lederle Laboratories Division of the American Cyanamid Company; Parke, Davis and Company; and the Upjohn Company.

with 0.1 cc. of 1:100 dilution of the culture and count plates were made at 1, 3, 5, 24, and 48 hours.

2. The susceptibility of the test strains to aureomycin was determined in the following manner. Serial two-fold dilutions of the antibiotic in Difco heart infusion broth, with 0.075 per cent dextrose added, were inoculated with equal volumes of 18-hour broth cultures so diluted as to result in an inoculum of approximately 200,000 organisms per cc. The tests were read at the end of 6, 12, 24, 48, 72, and 96 hours incubation at 37° C.

3. Drug deterioration was investigated in another set of experiments in which *K. pneumoniae* was the test organism. Three sets of tubes each containing two-fold dilutions of aureomycin from 0.625 to 20 micrograms per cc. were inoculated in the usual manner with the test strain. After 24 hours incubation growth was found to be inhibited by 2.5 micrograms per cc. of drug in all three sets of tubes. The first set (a) was allowed to incubate without further addition of drug through 72 hours. Fresh drug, to give an additional 2.5 cc. micrograms per cc. per tube, was added to the second set (b) at 24 hours, and to the third set (c) at 24 and 48 hours.

4. The activity of aureomycin in the presence of defibrinated, whole blood, fresh serum and heat-inactivated serum was determined in the following manner. Twenty-five hundredths cc. of blood or serum was introduced into each of 6 sterile pyrex tubes. Aureomycin, diluted in heart infusion broth to give the final concentrations desired, was added in 0.1 cc. amounts. Finally, 0.05 cc. of each ten-fold serial dilution (10^{-1} to 10^{-6}) of an 18-hour broth culture of the test organism was also added to each tube. The tubes were sealed, placed in a rotating box, and incubated at 37° C. for 24 hours, after which the tubes were opened and the contents cultured on blood agar plates. To determine the number of organisms added to each tube of blood or serum, count plates were made of the 10^{-5} and 10^{-6} dilutions. Control experiments were set up in the same fashion except that broth was used as the substrate rather than blood or serum.

Results

1. The growth curve, shown in FIGURE 1, indicates that although aureomycin brought about a progressive decline in the bacterial population for the first 24 hours of exposure, the organisms eventually recovered and attained growth equal to that in the control culture. Penicillin, on the other hand, caused an immediate decline in the bacterial count which terminated in the sterilization of the culture.

2. Whether or not the eventual outgrowth of organisms in the presence of aureomycin is the result of the survival of resistant organisms or is due to a deterioration of the drug is not known at present. That drug deterioration is an important and perhaps the most significant factor is illustrated in FIGURE 2. When aureomycin was titrated, in the manner already described, against *K. pneumoniae* as the test organism, the visible end-point shifted with progressive incubation so that at 6 hours the end-

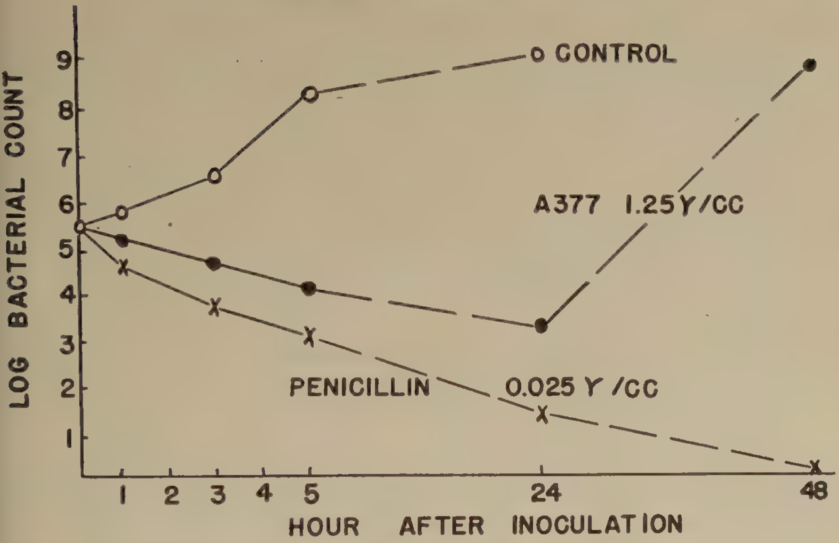

FIGURE 1. Effect of aureomycin and penicillin on the multiplication of C203.

point was 0.3 microgram per cc.; at 12 hours it was 1.25 micrograms per cc.; at 24 hours it was 5.0 micrograms per cc.; at 48 hours, 10 micrograms per cc.; at 60 hours, 20 micrograms per cc.; and at 72 hours it was greater than 20 micrograms per cc. Since repeated tests with this organism always titrated to essentially the same end-point at any given time, a six-hour test was developed for assaying the content of aureomycin in the body fluids of patients to whom the drug was being administered therapeutically. In each instance *K. pneumoniae* was employed as the test organism and a variety of specimens of blood sera and urines were assayed, the results of which will be reported in a later paper in this series.

Confirmatory evidence of drug deterioration was obtained in another set of experiments. The manner in which these experiments were performed has been described (see *Methods 3*), and the results are shown in FIGURE 3. In three identical sets of tubes containing two-fold dilutions of aureomycin from 0.625 through 20 micrograms per cc.—all inoculated at the same time—growth was found to be inhibited by 2.5 micrograms per cc. in all three sets of tubes at the end of 24 hours incubation. With fresh drug added to the second set (b) at 24 hours, growth was held down to 2.5 micrograms through 48 hours and then titrated to more than 20 micrograms at 72 hours. In set (c), with drug replenished at both 24 and 48 hours, the original titer of 2.5 micrograms per cc. was maintained throughout the 72-hour observation period. These results are in marked contrast with set (a) where, with no replacement of drug, growth titrated to 20 micrograms at 48 hours and to more than 20 micrograms at 72 hours.

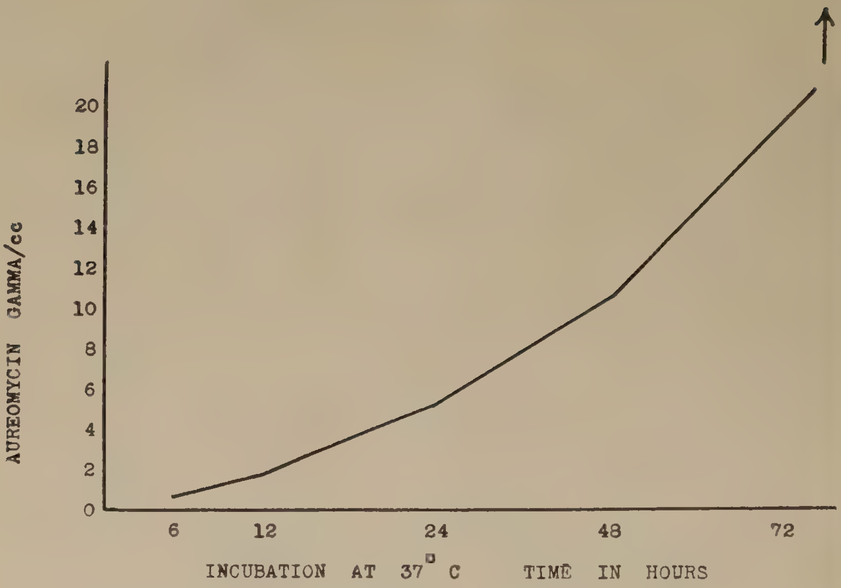

FIGURE 2. Shift in titer of aureomycin with progressive incubation. Test organism *K. pneumoniae*.

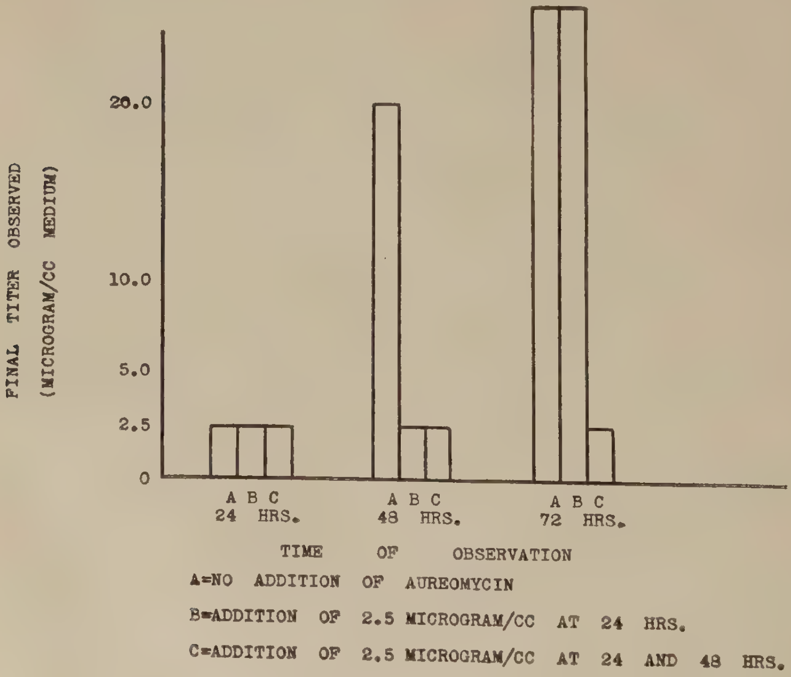

FIGURE 3. Effect of adding increments of fresh aureomycin.

That pH and temperature of the environment are significant factors in determining the rate of deterioration was shown by observing the end-points obtained with *E. coli* in solutions of aureomycin at different pH's held at −10° C. and at 37° C. for 23 hours previous to inoculation. The results are shown in TABLE 1. It can be seen that the deleterious effect of heat was augmented by an increase of pH.

TABLE 1

EFFECT OF THE TEMPERATURE AND pH OF STORAGE ON THE DETERIORATION OF AUREOMYCIN. TEST ORGANISM: *E. coli*; 24 HR. READINGS

Storage temperature	*Storage pH*			
	5.1	5.8	7.1	8.2
	Minimal inhibitory concentration—γ/cc			
−10° C.	3.12	3.12	6.25	6.25
37° C.	6.25	6.25	50.0	50.0

3. Studies on the activity of aureomycin in the presence of whole blood and serum are summarized in TABLES 2 and 3. When a *Staphylococcus aureus* was used as the test strain, as shown in TABLE 2, there was no indication that the bacteriostasis achieved with aureomycin alone was augmented by the bactericidal activity of whole blood or of serum, either fresh or inactivated. On the contrary, both blood and serum had a somewhat neutralizing effect on the action of the drug. This is evidenced by

TABLE 2

BACTERICIDAL STUDIES

Organism	*Substrate*	*Minimum number of organisms required to initiate growth/cc. of blood or serum*			
		Aureomycin gamma/cc.			
		0	5	10	20
Staphylococcus aureus	Whole blood	640	64,000	100,000	100,000
	Serum (fresh)	800		8,000	800,000
	Serum (inactivated)	800		80,000	800,000
	Broth (control)	1,600	1,600,000	1,600,000	1,600,000

TABLE 3

BACTERICIDAL STUDIES

Organism	*Substrate*	*Minimum number organisms required to initiate growth/cc. of blood or serum*		
		Aureomycin gamma/cc.		
		0	10	20
K. pneumoniae	Whole blood	3000	30,000,000	>30,000,000
	Serum (fresh)	4000	40,000,000	>68,000,000
	Serum (inactivated)	680	68,000	>68,000,000
	Broth	800	80,000,000	>80,000,000

the fact that, in the presence of 20 micrograms of aureomycin per cc., only 100,000 organisms were required to initiate growth in whole blood and 800,000 in serum, whereas in broth alone, 1,600,000 organisms were needed to produce growth. In this type of bacteriostatic test, there was no significant difference between fresh and inactivated serum with this organism.

Essentially the same results were obtained when *K. pneumoniae* was employed as the test strain, as shown in TABLE 3. In the presence of 20 micrograms of aureomycin per cc., more than thirty million organisms were required to initiate growth in whole blood, more than sixty-eight million in serum, but in broth alone, the minimal inoculum was eighty million. Again, the somewhat antagonistic effect of blood and serum on the drug is indicated by the above results. It is interesting to note that in the presence of 10 micrograms of drug per cc., fresh serum was more bactericidal than inactivated serum against *K. pneumoniae*. This is shown by the fact that forty million organisms were required to initiate growth in fresh serum as compared with only sixty-eight thousand organisms in heat inactivated serum. The most likely explanation for this observation is that the bactericidal effect of fresh complement on a gram negative organism tends to offset the neutralizing effect of serum itself on the drug. That this explanation is probable is suggested by the finding that neither fresh nor inactivated serum had any bactericidal effect in the absence of drug.

Discussion

The performance of aureomycin in the test tube is not brilliant when compared with that of polymyxin or penicillin. When due consideration is given to the fact that this antibiotic deteriorates rapidly under experimental conditions, it is surprising that aureomycin appears to be much more promising clinically, even at minimal blood levels, than it does experimentally. One is led to suppose that the mode of action in the body may be different from that *in vitro*.

In spite of the antagonistic effect of whole blood and serum, it is significant that organisms are actually killed in these substrates when sufficient quantities of aureomycin are present.

Summary

1. The new antibiotic, aureomycin, is effective *in vitro* against both gram negative and gram positive bacteria. Higher concentrations of this drug are required to produce bacteriostasis than are necessary with either polymyxin or penicillin, being close to those required of streptomycin.

2. Aureomycin is bacteriostatic rather than bactericidal in its effect.

3. The drug deteriorates rapidly at room and incubator temperatures in neutral or alkaline solution, but bacteriostasis can be maintained if fresh drug is added every 24 hours. Since the rate of deterioration is

fairly constant for any given test organism, a rapid assay method can be used for determining the content of aureomycin in body fluids.

4. The activity of aureomycin is not enhanced by the presence of whole blood or serum. On the contrary, although actual killing takes place if sufficient drug is present, both blood and serum exert an antagonistic effect on the activity of this antibiotic.

LABORATORY STUDIES WITH AUREOMYCIN[*]

By THOMAS FITE PAINE, JR.,[†] HARVEY SHIELDS COLLINS,
AND MAXWELL FINLAND

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SOME of the results of the studies carried out by the workers of the Lederle Laboratories which were made available to us suggested that aureomycin, a new antibiotic, was relatively non-toxic and highly effective, particularly against certain viral and rickettsial infections in animals, and seemed worthy of clinical trials. The studies reported here were carried out as a part of the evaluation of the clinical effect of this agent in the treatment of certain human bacterial infections.

Sensitivity of Various Bacteria. Tests for sensitivity were performed on 186 strains of organisms recently isolated from patients. Tube-dilution and streak-plate methods were used for these tests. The strains of *Diplococcus pneumoniae*, *Neisseria gonorrhoeae*, *N. meningitidis* and *Streptococcus pyogenes* were all inhibited by 1 μg. or less of aureomycin per ml. Staphylococci and most strains of gram negative bacilli, including species of *Salmonella*, were inhibited for the most part by concentrations of aureomycin in the range of 1 to 25 μg. per ml. Strains of only 2 species, namely *Proteus vulgaris* and *Pseudomonas aeruginosa*, were moderately resistant to the action of aureomycin, requiring from 125 to 250 μg. per ml. for complete inhibition.

Comparison with Other Antibiotics. No evidence of cross-resistance with other antibiotics was found. Penicillin- and streptomycin-sensitive and resistant strains of the same organism were found to be equally sensitive to the antibacterial activity of aureomycin. On a weight basis, aureomycin was found to be less effective than penicillin, but more effective than streptomycin in inhibiting coccal organisms and was about equally as effective as streptomycin against most gram negative bacilli.

Stability. Aureomycin hydrochloride as provided to us was found to maintain its potency on storage at room temperature in the dry powder in sealed ampules for at least 7 months. It also retained most of its activity when kept in solution in high concentrations in distilled water at 37°

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[*] Aided by a grant from the United States Public Health Service.

Generous supplies of aureomycin hydrochloride and some of the data concerning its activity *in vitro* and in experimental animals were supplied by the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York.

The laboratory studies were carried out with the technical assistance of Clare Wilcox, Janice M. Bryan and Paul F. Frank. The strains of pathogenic bacteria used in these studies were isolated and identified by Marion E. Lamb and A. Kathleen Daley in the Bacteriology Laboratory of the Mallory Institute of Pathology and by Helen Trousdale in the Urological Clinic, Boston City Hospital.

Dr. E. Buist Wells participated in some of the pharmacologic studies.

[†] Research Fellow, American College of Physicians.

C. or at 4° C. In low concentrations in broth, plasma or blood agar, however, a rapid decrease in potency occurred at 37° C., and a somewhat less rapid fall in potency took place at 4° C.

Comparison of Tube-dilution and Streak-plate Methods for Determining Sensitivity of Bacteria

Effect of Size of Inoculation. Bacterial sensitivities to aureomycin could be performed satisfactorily using either a tube-dilution technique or by streaking cultures directly on the surfaces of a series of agar plates containing graded concentrations of the antibiotic. The inoculum size influenced the concentration of aureomycin required for complete inhibition when using the tube-dilution method, but did not alter the inhibition end-points when the surface streak method of determining sensitivities was used. Aureomycin did not, apparently, exert antibacterial activity against fully grown cultures of microorganisms. However, against vigorously multiplying organisms, it exerted considerable antibacterial activity.

Effect of pH. The pH of the media exerted considerable influence on the inhibition end-points observed in the tube-dilution sensitivity tests. Increasing the pH from 6.1 to 8.0 resulted in a 64-fold increase in the concentration of the antibiotic required to inhibit one of the organisms tested.

"Aureomycinase"? There was no evidence of the elaboration of any aureomycin-inhibiting substance (similar to penicillinase) by any aureomycin-resistant organisms, nor could any such material be extracted from the disrupted bacterial cells of a number of organisms.

Effect of Aerobiosis. The degree of aerobiosis may affect the inhibition end-points of aureomycin against certain organisms. Preliminary experiments suggested that a marked reduction in oxygen content in the environment of a culture resulted in some diminution in the antibacterial activity of aureomycin. The activity of aureomycin on cultures that were incubated in a candle jar, however, was not diminished.

Development of Resistance IN VITRO. The bacteria studied showed no marked tendency to become resistant to aureomycin on repeated exposure to the drug, though an occasional strain did increase in resistance after many subcultures in increasing concentrations of the antibiotic. No evidence could be found for the development of aureomycin "dependent" strains of bacteria. Resistant variants could not be isolated readily by exposure of large bacterial populations to high concentrations of the antibiotic.

Absorption and Excretion

Blood Levels. Many attempts were made to determine the blood concentrations of aureomycin in patients receiving this antibiotic by assaying the serum against selected test strains of bacteria. Despite doses up to 2.0 grams daily by mouth, it was not possible to inhibit a test strain of

Streptococcus which was sensitive to 0.5 μg./ml. in serum dilutions beyond 1 : 2 or occasionally 1 : 4. With Bacillus No. 5 (a *subtilis*-like organism inhibited by 0.2 μg. of aureomycin per ml.), it was possible to obtain inhibition in serum dilutions up to 1 : 8 or 1 : 16.

Urinary Excretion. Following oral or intramuscular doses of aureomycin, the material appeared in the urine within the first hour and the maximum amount appeared during the following 4 to 8 hour period. Following single doses of 0.5–0.75 gm. by mouth, as much as 13.4 per cent of the dose could be recovered in the urine during the following 55 hours. The maximum concentration in the urine on oral doses of 1-2 grams daily ranged up to 256 μg. per ml. An interesting feature was the prolonged excretion of the drug, following oral doses. Antibiotic activity was demonstrated in urine collected after three, and sometimes after four days following the last oral dose. In patients receiving repeated doses for severe infections, the total amount recovered from the urine was only 3-5 per cent of the amount administered, but technical difficulties may have accounted, in part, for the failure to recover larger proportions of the administered drug.

Summary

Aureomycin possesses antibacterial activity against many microorganisms, including coccal and bacillary forms. It is active against certain penicillin-resistant organisms as well as against streptomycin-resistant and dependent strains. It is stable in the dry powder at room temperature and in high concentration in solution in distilled water at 37° C. or 4° C. Loss of activity occurs rapidly when the antibiotic is incubated at 37° C. in low concentration in broth, plasma or blood agar, though the loss in activity in the same media is less rapid on refrigeration.

The antibiotic is most active in the acid pH range. The number of organisms present, as well as their growth phase, influences the inhibition end-points observed in the tube-dilution method of sensitivity determination.

Bacteria do not readily become resistant to aureomycin *in vitro*, though such an alteration was achieved in the case of certain microorganisms.

On oral doses of aureomycin of 1 or 2 grams daily in adults, concentrations up to 256 μg. per ml. may be found in the urine. Excretion of the antibiotic in the urine continues for 3 days or longer after its administration.

CLINICAL STUDIES WITH AUREOMYCIN*

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SOME of the preliminary findings on the toxicity of aureomycin and its effect in experimental infections that were made available to us by the workers of the Lederle Laboratories suggested that this agent was relatively non-toxic, was effective by mouth, and gave promise of being highly active against certain viral and rickettsial infections. Studies *in vitro* by the same workers, which were confirmed and extended in this laboratory, also indicated that it was effective against a wide range of human pathogenic bacteria. This new antibiotic, therefore, seemed worthy of clinical trials in bacterial infections in order to determine its range of usefulness.

Certain laboratory studies which were carried out as part of the clinical trials are reported separately.[1, 2] To date, about 100 patients with a variety of bacterial infections have been treated with aureomycin by mouth at the Boston City Hospital. The results obtained in these patients are summarized in this communication.

Infections Treated and General Results

The types of infection treated, the dosage used, and a rough estimation of clinical effect of aureomycin in these cases are listed in TABLE 1. It should be borne in mind that most of these patients were treated before there was much knowledge of the toxicity of the drug in humans and the dosage employed was gradually increased in successive patients until considerably greater amounts were used than any that had previously been given by mouth. These patients received no other chemotherapy or antibiotic during the aureomycin treatment, although many of them had been treated unsuccessfully with such agents before the aureomycin was started.

Good results were obtained in most of the coccal infections. The effects in most of the patients with typhoid fever, *Salmonella* infections and severe urinary tract infections were difficult to evaluate. In most of the patients in whom the results are listed as doubtful, definite clinical and bacteriological evidence of beneficial effects was noted during treatment with aureomycin, but the results were either transient or the role of the antibiotic in effecting the results could not be evaluated.

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* Aided by a grant from the United States Public Health Service.

† Research Fellow, American College of Physicians.

TABLE 1

SUMMARY OF RESULTS OF AUREOMYCIN TREATMENT IN 102 PATIENTS

		Total therapy, by mouth		*Results*		
Diagnosis	*Patients*	*Grams*	*Days*	*Good*	*Doubtful*	*Failed*
Gonorrheal urethritis, male	66	1–3	1–2	49	11	6
Pneumococcal pneumonia	4	5–20	5–10	4	0	0
Meningococcemia, acute	1	4	3	1	0	0
Typhoid fever	5	3–39	13–22	1	2	2
Typhoid carrier	1	23	31	0	0	1
Acute enteritis, *S. newport*	2	5–7*	3–8	1	0	1
S. suispestifer bacteremia (vertebral and psoas abscesses)	1	21	11	0	0	1
E. coli bacteremia	1	22.5	15	0	0	1
Cystitis and pyelonephritis	16†	3–28	3–28	6	15	2
Lymphogranuloma venereum	1	40	20	0	1	0
Measles	1	1	2	0	0	1
Relapsing fever (?)	1	9–11	5–6	0	0	1
Non-specific urethritis	2	4	7	2	0	0
Total	102			64	29	16

* One of these patients also received 20 mg. intramuscularly twice a day.

† Seven of these patients received 2 separate courses because of reinfection with new strains and are each listed twice under "Results."

Suitable cases of rickettsial infections or of viral diseases other than those listed were not available for treatment during the study. A brief résumé will be given of the results in certain groups of cases.

Gonococcal Urethritis. The cases of acute gonococcal urethritis were all in males, and were treated in the Out-Patient Clinic. The results with the various dosage schedules that were tried are shown in TABLE 2. Failures were most frequent among the patients who were treated for only one day and with a total dose of only 1 or 1.5 gm. of aureomycin. In them, urethral discharge was present and contained numerous well-formed leucocytes on the day following treatment. This was true even among those listed as "Cured" and even when cultures and smears of this discharge were negative for gonococcus. Complete subsidence of the discharge in these cases occurred in 2 or 3 days. The patients who received doses on 2 successive days showed a response more like that which usually follows an adequate dose of penicillin. In them, the discharge cleared rapidly and leucocytes, when present on the day after treatment ended, looked disrupted and stained poorly.

It is thus evident that aureomycin was effective against acute gonorrhea in the males. The over-all results in this group of cases, however, are distinctly inferior to those observed in this same clinic in patients treated with 300,000 units of penicillin given intramuscularly in a prolonging agent. The optimum dosage schedule of aureomycin, however, has not yet been defined, and the effects of intramuscular therapy were not explored.

TABLE 2

SUMMARY OF RESULTS IN CASES OF ACUTE GONOCOCCAL URETHRITIS IN MALES TREATED WITH AUREOMYCIN BY MOUTH*

(Boston City Hospital—to July 9, 1948)

	Aureomycin				
Number of patients	*Schedule 0.5 gm. doses*	*Total gm.*	*"Cured"*	*Failed†*	*No follow-up*
9	10 a.m. & 10 p.m.	1.0	4	3 (1)	2
21	10 a.m.; 5 & 10 p.m.	1.5	17	3 (1)	1
5	10 a.m.; 2, 6 & 10 p.m.	2.0	4‡	0	1
21	10 a.m. & 10 p.m. (day 1); 10 a.m. & 10 p.m. (day 2)	2.0	14	3 (1)	4
8	10 a.m.; 5 & 10 p.m. (day 1); 10 a.m. & 10 p.m. (day 2)	2.5	8	0	0
1	10 a.m. & 10 p.m. (day 1); 10 a.m. (day 2)	1.5	1	0	0
1	10 a.m.; 2, 6 & 10 p.m. (day 1);** 10 a.m. & 10 p.m. (day 2)	3.0	1	0	0
66			49	9 (3)	8

* Bacteriological studies carried out by Mrs. Helen Trousdale; the clinical studies were carried out through the courtesy of Dr. George C. Prather, Chief of the Urological Service, with the assistance of Drs. Thomas F. Kaiser and Francis C. Regan.

† In parentheses are shown the number of patients, included among the failures, who had delayed reappearance of symptoms and positive cultures after having completely cleared; they may represent reinfections.

** Discharge present and culture positive at 9 a.m. on day 2.

‡ One patient had acute orchitis which subsided promptly.

Pneumococcal Pneumonia. Four patients with pneumococcal pneumonia were treated with aureomycin by mouth. The relevant findings are summarized in TABLE 3. In 3 of the patients, including one with bacteremia, treatment was begun on or before the second day of illness. In each of these patients improvement, both subjective and objective, occurred rapidly and they were all afebrile within 18 to 36 hours. In patient B. C., who had a very severe infection with jaundice, treatment with aureomycin was started on the fourth day. Improvement in this patient was somewhat more gradual and the temperature dropped to normal within 48 hours.

Careful bacteriological studies in these 4 patients revealed a rapid decline in the number of pneumococci in the sputum following the first dose of aureomycin and no pneumococci could be recovered in any of them after 48 hours. The results are similar to those obtained with full doses of penicillin in comparable patients.

The various organisms isolated from the sputum in patient J. T. and the sensitivity of these organisms to aureomycin are listed in TABLE 4. Only alpha hemolytic streptococci could be isolated after the second day of treatment.

Meningococcemia. The patient with meningococcemia is of interest because treatment with aureomycin was started within 12 hours of the onset of an illness which was tentatively diagnosed as Rocky Mountain spotted fever. The patient had been in an endemic focus and had had tick

TABLE 3

RELEVANT FINDINGS IN 4 PATIENTS WITH PNEUMOCOCCAL PNEUMONIA TREATED WITH AUREOMYCIN BY MOUTH

						Dosage			
Patient	*Age*	*Type*	*Blood culture*	*Lobes**	*Severity*	*Schedule*	*Total gm.*	*Day treatment begun*	*Hours of fever after first dose*
PG	19	2	0	LL	++	0.5 gm. + 0.25 Gm. Q 6 Hr.	5.	2	36
BC	65	7	0	LL	+++	0.5 gm. Q 6 Hr.	20.	4	48
SW	23	4	0	RL	++	0.5 gm. Q 8 Hr.	10.5	1	18
JT	16	44	+	RL	+++	0.5 gm. Q 6 Hr.	10.	2	18

* LL = left lower; RL = right lower.

TABLE 4

SENSITIVITY TO AUREOMYCIN OF STRAINS OF ORGANISMS ISOLATED FROM A CASE OF PNEUMOCOCCAL PNEUMONIA*

Date	*Organism*	*Inhibiting concentration of aureomycin*	
		Complete *µg./ml.*	*Partial* *µg./ml.*
June 27	*S. mitis*	6.3	3.1–1.6
27	*S. aureus*	3.1	1.6–0.8
27	*S. pyogenes*	0.8	0.4–0.1
27	*D. pneumoniae*, type 44	3.1	1.6–0.8
27	*D. pneumoniae*, type 44‡	3.1	1.6
28	*S. mitis*	3.1	1.6
28	*S. aureus*	3.1	1.6–0.8
28	*D. pneumoniae*, type 44	3.1	1.6–0.4
28	*N. catarrhalis*	3.1	1.6–0.4
29	*S. mitis*	12.5	6.3–3.1
30	*S. mitis*	12.5	6.3–3.1

*Patient J. T.; aureomycin, 0.5 gm. every 6 hours started on June 27 after sputum and blood were obtained for culture.
‡ Organism from blood culture; all others from sputum.

bites and ticks removed from her body 7 to 10 days before the onset of headache, severe stiff neck, and a rash. The spinal fluid was entirely negative. Symptomatic improvement was rapid and the patient was afebrile within 18 hours of the first dose. An initial blood culture was positive for group I meningococcus. Subsequent blood cultures were all negative.

Typhoid Fever, SALMONELLA *Infections, and Colon Bacillemia.* Of the 5 cases of typhoid fever, 4 were in children under 5 and one was in a man 60 years old. Treatment in the children was begun between the 5th and 10th day of illness. They all had positive blood cultures before aureomycin was started, but those taken after 1 or 2 days of therapy were all sterile. In the older patient, treatment was begun 4 days after a relapse with severe symptoms. Bacteremia in this patient persisted during 6 days of aureomycin therapy. The cultures of urine and stools in all of the 5 patients were negative for *S. typhosa* after the second day of treatment. Isolation of *Salmonella* from stools obtained during aureomycin treatment, however, was difficult, because the cultures were frequently overgrown with *Proteus*. Symptomatic improvement occurred soon after treatment was started in only 1 of these 5 patients and was more gradual in the others. Evaluation of the effect of the drug in these patients with typhoid fever was very difficult.

The illness in each of the 3 patients with the other *Salmonella* infections was very severe. Clinical and bacteriological improvement occurred in only one of them in relation to the onset of treatment. One of the 2 patients with *S. newport* infection and the patient with *S. suispestifer* bacteremia died, and the *Salmonella* persisted in these 2 patients throughout 3 and 11 days, respectively, of treatment with aureomycin, 0.5 gm. every

6 hours by mouth and 20 mg. intramuscularly twice a day. Autopsy revealed no focus in the former, and a large vertebral and psoas abscess which yielded *S. suispestifer* was found in the latter.

A patient with a persistent colon bacillus bacteremia and evidence of infection and possibly of malignancy in the abdomen showed temporary symptomatic improvement during treatment with aureomycin by mouth. The bacteremia recurred, however, and the patient died after an intestinal hemorrhage. There was no autopsy.

The relevant bacteriological findings in these 9 patients are summarized in TABLE 5.

TABLE 5

BACTERIOLOGICAL FINDINGS IN CASES OF *Salmonella* INFECTIONS AND IN COLON BACILLEMIA TREATED WITH AUREOMYCIN

			Positive cultures in relation to aureomycin therapy				
Patient	*Organism*	*Sensitivity μg./ml.*	*Source*	*Before*	*During*	*After*	*Clinical result*
R.H.	*S. typhosa*	12.5	Blood	+	0	0	Good
			Stool	+	0	0	
J.H.	*S. typhosa*	12.5	Blood	+	—	—	Doubtful
			Stool	+	+ 1 day	0	
L.O.	*S. typhosa*	12.5	Blood	+	+ 1 day	—	Good ?
			Urine	0	0	—	
			Stool	0	0	—	
M.O.	*S. typhosa*	25	Blood	+	0	—	No effect?
			Urine	0	0	0	
			Stool	0	0	—	
A.L.	*S. typhosa*	6.3	Blood	+	+ 6 days	—	No effect?
			Urine	0	0	0	
			Stool	+	0	0	
M.E.	*S. typhosa* (chronic carrier)	12.5	Stool	+	+ 8th day	+	No effect
			Duodenum	—	+ 30th day	—	
P.E.	*S. newport*	25	Blood	0	0	0	Good
			Urine	0	0	—	
			Stool	+	0	0	
C.M.	*S. newport*	6.3	Blood	0	—	+ A*	Died
			Urine	+	—	+ A	
			Stool	+	+	+ A	
A.A.	*S. cholerae suis*	6.3	Blood	+	+	+ A**	Died
			Urine	0	—	—	
F.S.	*E. coli*	12.5	Blood	+	+	—	Died

* A = Autopsy culture.
** Same organism from vertebral and psoas abscess and from liver at autopsy.

Urinary Tract Infections. The cases of urinary tract infections that were treated with aureomycin were all severe. Seven of the 16 included in this study received a second course of a larger amount of aureomycin by mouth after an interval of about 2 weeks. All but one of these 16 patients were known to have urinary tract infections longer than one month, and in some instances, the infection had persisted for many years. In almost every instance there was a prolonged history of complicating factors such as urinary retention, frequent instrumentation, prostatectomy, urolithiasis, and chronic or recurrent acute cystitis and pyelonephritis. One or more chemotherapeutic or antibiotic agents had previously been used in most of these patients without producing prolonged remissions.

Only one of the patients had acute pyelonephritis and had received no previous treatment. It is obvious, therefore, that the severest and the least favorable types of cases were chosen for an evaluation of the efficacy of aureomycin.

The predominant clinical symptoms in these cases were dysuria, poor sphincter control, nocturia, frequency and cloudy urine. The cultures obtained before aureomycin treatment usually revealed pure cultures of a single strain. In most patients who received a second course of aureomycin, organisms other than those initially present had appeared either during or after the first course.

Aureomycin was given, usually for 7 days, in doses of 0.5 gm. morning and evening. Those who were retreated received 0.5 gm. 4 times a day. The effect of still larger doses is now being studied. Only 4 of these patients were hospitalized during treatment.

In general, pyuria diminished markedly during therapy and disappeared entirely in about half of the cases while treatment was still being given. Symptomatic relief occurred at the same time in most instances; the burning on urination, nocturia and frequency all diminished. The urines became sterile during treatment in most of the patients. In some of the patients *Proteus* and *Pseudomonas*, and in 2 instances *E. coli*, either first appeared or persisted during aureomycin treatment. Infection recurred within a few days in most instances, with either the same or with different types of organisms. The most persistent organisms were the *Proteus* and, indeed, these seemed almost to flourish, but *Pseudomonas* also could not be eradicated.

Good results of a permanent nature were, therefore, infrequent in these cases of chronic urinary tract infections. Temporary relief, either slight or moderate, resulted in two-thirds of the cases.

A summary of the bacteriological findings, including the aureomycin sensitivity of the various strains isolated, is given in TABLE 6. It is seen

TABLE 6

ORGANISMS ISOLATED FROM CASES OF URINARY TRACT INFECTIONS TREATED WITH AUREOMYCIN

Organism	No. of pts.†	Sensitivity μg./ml.	*Number of cases* Before Rx	During Rx	After Rx
Streptococcus mitis	1	6.3	1	0	0
Klebsiella pneumoniae	1	50	1	1	
Aerobacter aerogenes	9^1	6.3–100	7	4	3*
Escherichia coli	9^2	6.3–100	11	6	3
Pseudomonas aeruginosa	4^1	200–250	3	3	4
Proteus vulgaris	7^4	125–250	4	8	9

* Two of these were the only strains of this organism resistant to 50 or 100μg./ml.

† Superscripts indicate number of cases included in which the same organism was isolated in relation to 2 separate courses of therapy.

that the more resistant organisms, particularly *Proteus* and *Pseudomonas*, were the ones which persisted throughout treatment or appeared after treatment with aureomycin.

Lymphogranuloma Venereum. One patient with bilateral inguinal adenopathy of two weeks' duration and a positive Frei test was given a total of 40 gm. of aureomycin by mouth over a period of 20 days. The inguinal nodes on the right had enlarged progressively and became increasingly tender during the previous 2 weeks, and 2 days before treatment began to discharge foul pus which was bacteriologically sterile. During the aureomycin therapy, drainage and tenderness on that side diminished markedly within 4 days, and subsided almost completely after 2 weeks. The sinus tract persisted and looked clean and the swelling regressed considerably. The nodes on the left increased in size and in tenderness during the treatment; they became fluctuant by the 11th day, and were aspirated on that day and again on the 18th day. There was progressive diminution in the swelling and tenderness, but no sinus tract developed. After aureomycin was discontinued, sulfadiazine was given for 5 days without evidence of any further improvement.

Measles. A 4-year-old child was given 1 gm. of aureomycin during the third day of fever and first day of rash. Fever persisted at 104° to 105° and the drug was discontinued because of looseness of the bowels and prostration. The course in this child was identical to that of his brother, who was not treated.

Non-specific Urethritis. Two patients were referred by Dr. Howard M. Trafton, because of persistent urethritis in spite of repeated therapy with penicillin, sulfonamides, and streptomycin. Cultures were negative for *Gonococcus* and in one of the patients a pleuropneumonia-like organism was isolated. In the latter patient, a total of 9 gm. of aureomycin was given by mouth over a period of 6 days. The patient was symptomatically improved within 3 days and the discharge stopped and only occasional cells could be seen in the prostatic smears. Cultures were negative after treatment. The other patient was given 11 grams in 5 days. He became asymptomatic quite rapidly. The discharge began to clear before the treatment was ended and was completely gone within 11 days.

Toxicity. Significant toxic effects were notably absent. Doses of 250 mg. or less, twice a day, were used in adults at first, but these have been increased progressively until recently an initial dose of 4 gm. with daily doses of 2 gm. have been used in many cases. The treatment has been maintained in most patients, except those with gonorrhea, for 1 to 2 weeks on this dosage and as long as one month on a dose of one gram a day. Minor symptoms were more frequent with the higher doses.

The commonest complaint during oral therapy with aureomycin was looseness of the bowels with frequent, bulky, and soft stools. True diarrhea was uncommon. Normal bowel action again occurred when the drug was discontinued or when the dose was temporarily decreased. Nausea and, occasionally, vomiting occurred after one or more doses in a few patients but could not always be related to the treatment. In some of the patients with cystitis, a disagreeable sensation of as something "drawing" or "squirming" in the pelvis was noted. This may be related to the high acidity of the urine during ingestion of large doses of aureomycin. Other symptoms were not noted.

The patients treated in the hospital were followed carefully with frequent blood counts and in some of them liver function tests were done. Anemia ascribable to the drug did not occur. No depression of the cells in the granulocytic series was observed. There was no evidence of renal irritation as manifested by rising non-protein nitrogen levels in the blood, albuminuria or appearance of cellular elements in the urine. Jaundice did not develop in any of the patients, nor was there any evidence of liver impairment developing after treatment was started. No rashes or fever ascribable to the drug were observed. In a 3-year-old patient with typhoid fever, fever recurred and increased after the temperature had almost reached normal. The patient remained otherwise well and was, therefore, suspected of having drug fever. A single 200 mg. dose given after the child again was afebrile for a few days produced no fever or symptoms, so that the relation of the drug to the persistent fever could not be evaluated.

Intramuscular doses were used in only 3 patients. In the first of these patients, the injection of 20 mg. caused considerable local pain, but in the others, who were severely ill and were given similar amounts repeatedly for several days, there was no apparent discomfort. Sterile saline was used as a diluent in these latter cases.

Bacterial Resistance. Studies of the sensitivity of the organisms which were obtained in the same patients before, during, and after treatment with aureomycin yielded no evidence of the development of bacterial resistance to aureomycin *in vivo*.

Conclusions

Aureomycin by mouth in the doses used exerted a definite beneficial effect in the coccal infections. The results in the cases of gonorrhea, however, were inferior to those obtained with adequate amounts of penicillin. In the urinary tract infections, when the bacteriological findings and the type of cases chosen for treatment are taken into consideration, the results were favorable and may be comparable with, or possibly superior to those resulting from the use of streptomycin in similar cases. Infections with *Proteus vulgaris* and *Pseudomonas aeruginosa*, however, were not benefited by aureomycin. The results in the cases of typhoid fever and of severe *Salmonella* infections were equivocal. The only evidence of toxicity was the occurrence of copious, loose bowel movements when large doses were given by mouth. Resistant organisms did not develop during treatment with this antibiotic.

References

1. Paine, T. F., H. S. Collins, & M. Finland. 1948. Laboratory studies with aureomycin. Ann. N. Y. Acad. Sci. **51**(2): 228–230.
2. Collins, H. S., E. B. Wells, T. F. Paine & M. Finland. Urinary excretion of aureomycin. Preliminary report. Proc. Soc. Exp. Biol. & Med. (In press.)

STUDIES ON SERUM CONCENTRATIONS IN HUMANS AND PRELIMINARY OBSERVATIONS ON THE TREATMENT OF HUMAN INFECTIONS WITH AUREOMYCIN*

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AUREOMYCIN,* a new antibiotic, which has been found to be effective *in vitro* and in animals against a variety of microorganisms, has been employed by us in human infections. In the present paper, we are reporting the results of preliminary studies, which include: (1) The determination of the concentration of the antibiotic in the blood after oral and intramuscular doses, (2) observation of patients for toxic reactions after single and multiple doses, and (3) clinical tests in a few patients.

Determination of Blood Aureomycin Concentrations. Attempts were made to determine the amount of aureomycin present in the serum with the use of the tube dilution method, making readings after twenty-four hours as in the determination of penicillin and streptomycin. It soon became apparent that the inhibiting effect of aureomycin upon bacteria was seldom present for as long as twenty-four hours. Incubation at 37° C. of 1 microgram per cc. or less of aureomycin, in tryptose phosphate broth, thioglycollate medium, or human serum, showed that only one-sixteenth to one-thirty-second of the original activity remained after eight hours. Neither the presence or absence of organisms, nor culturing under oil, made any difference in these results.

The effect of the antibiotic upon the growth of a strain of *Bacillus cereus*† is shown in FIGURE 1. This test was performed by placing in a series of tubes 3 cc. of a 1:10,000 dilution in tryptose phosphate broth of a 16-hour culture of *Bacillus cereus*. To one tube, 1 cc. of sterile broth was added and to other tubes, aureomycin in broth, to make a final dilution of 10, 2.5, 0.625, and 0.039 micrograms, respectively. The tubes were incubated at 37° C. and at 1, 2, 4, 6, 8, 12, and 24 hours after the start of incubation, 0.1 cc. of the mixture was withdrawn from each tube, diluted 1:1000 or 1:10,000, and plated after mixing with nutrient agar. The number of colonies on each plate were counted after twenty-four hours' incubation.

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* Supplied by the Lederle Laboratories Division, American Cyanamid Company.
† This strain was known as *Bacillus* No. 5 and was supplied by Mr. A. C. Dornbush

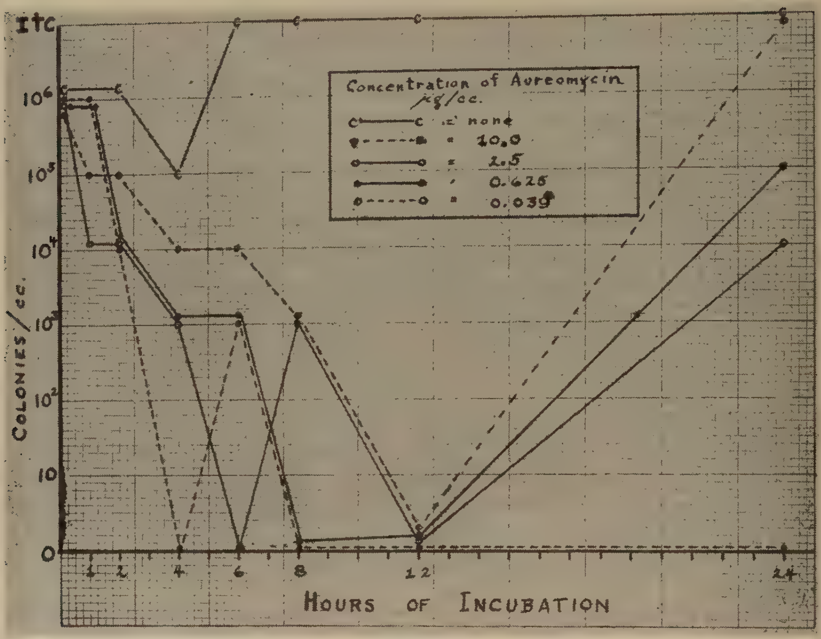

Figure 1. The effect of varying concentrations of aureomycin upon the growth of a strain of *Bacillus cereus*.

Figure 1 shows the result of a typical experiment. Plates taken from the control tube (without aureomycin) contained colonies too numerous to count (ITC) from the sixth hour on. The mixture containing 10 micrograms per cc. of aureomycin inhibited growth of all organisms from the eighth hour on, while those with smaller amounts of aureomycin showed inhibition of growth up to the twelfth hour and growth again by the twenty-fourth hour. In repeated experiments, the period of maximum depression of the bacterial count was found to be between the fourth and the twelfth hours.

From our data, therefore, it would appear that when a method of assay is used in which readings are made at twenty-four hours, only those concentrations are detected which are high enough initially to have complete bactericidal action before the aureomycin is inactivated, or which are high enough initially to retain some bacteriostatic action for twenty-four hours.

Since aureomycin is merely bacteriostatic in low concentrations, in determining serum concentrations in patients, we followed the method of Dornbush,[1] namely, incubation for four hours in a water bath. The only modification of his method was the use of tryptose phosphate broth.

Tables 1, 2, and 3 show the serum concentrations of aureomycin after the administration of single doses intramuscularly or by mouth. After

TABLE 1

NUMBER OF SPECIMENS AT EACH BLOOD CONCENTRATION AFTER ADMINISTRATION OF 100 MG. I.M.

Concentration	*Hours*						
	1	*3*	*6*	*8*	*12*	*18*	*24*
2.0		1					
1.0	2			1			
0.5		2					
0.25	3	1	1				
0.125					2		
0.06		1	4	1	3	1	1
0					1		5
Total	5	5	5	2	6	1	6

the intramuscular injection of 100 mg. to adults (TABLE 1), the peak concentration of the antibiotic was reached at about the third hour. Detectable concentrations were present in the blood stream at the twelfth hour in nearly all patients and, in the case of one patient, as long as twenty-four hours after injection.

When 700 mg. were given by mouth (TABLE 2), the peak concentration in the serum was reached around the sixth hour and the antibiotic was

TABLE 2

NUMBER OF SPECIMENS AT EACH BLOOD CONCENTRATION AFTER ADMINISTRATION OF 700 MG. P. O.

Concentration	*Hours*						
	1	*3*	*6*	*8*	*12*	*18*	*24*
4.0			1				
2.0		2		2			
1.0	2	2	5	1	1		
0.5	3		2				
0.25		3		2			
0.125	3	1	1		2		
0.06					2	1	
0						2	3
Total	8	8	9	5	5	3	3

TABLE 3

NUMBER OF SPECIMENS AT EACH BLOOD CONCENTRATION AFTER ADMINISTRATION OF 300 MG. P. O.

Concentration	*Hours of administration*				
	1	*3*	*6*	*12*	*24*
1.0		2	2		
0.5	1	2	2		
0.25	2	1	1	4	
0.125	1				
0.06				1	3
0					2
Total	4	5	5	5	5

detectable in the sera of all patients tested at the twelfth hour. After the oral administration of 300 mg., the peak levels were not as high as with 700 mg., but aureomycin was still detectable in the serum of all the patients at twelve hours.

Five patients who received 10 mg. per kilogram of body weight at six-hour intervals showed no appreciable difference in serum concentrations on the third to the seventh day of treatment compared with the first day of treatment. Although the optimal dosage schedule still needs to be worked out, it appears that oral doses of 5 to 10 mg. per kilogram at six-hour intervals are sufficient to assure that measurable amounts of aureomycin will be consistently present in the blood.

Toxic Reactions. We have observed no untoward reactions to aureomycin, except pain at the site of intramuscular injection. This pain was present in all of our patients who were given intramuscular injections. The dose varied from 100 mg. to 1400 mg. The pain was an immediate stinging or aching sensation at the site of injection which lasted from 15 minutes to 2 hours. It was more intense and lasted longer when the larger doses were given. In no instance was there any delayed reaction or any generalized effect from the injections. We have given aureomycin intramuscularly to patients in doses of 5 to 1400 mg. and orally in doses of 100 to 700 mg. as shown in TABLES 4 and 5.

TABLE 4

NUMBER OF PATIENTS TREATED INTRAMUSCULARLY

Dose (mg.)		*Pt.*	*Days*
	Single doses		
100		7	
300		2	
350		3	
700		2	
1400		1	
	Multiple (daily) doses		
5, 10 & 20		1	3
40, 80, 160, 320		1	4
700, 300, 350		1	3

TABLE 5

NUMBER OF PATIENTS TREATED ORALLY

Dose (mg.)		*Pt.*	*Days*
	Single doses		
700		11	
350		1	
300		4	
	Multiple doses		
700 q6h		2	7
700 q6h		1	3
300 q6h		1	7
250 q6h		1	6

Observations on Patients Treated. Four patients with typhoid fever were treated. In two, the treatment was started during the second week and in two, during the third week of the disease. The patients received 10 mg. per kilogram of body weight orally every four hours. No effect upon the clinical course was observed. Blood cultures remained positive during treatment and, in one patient, the sensitivity of the organism changed from 0.6 micrograms per cc. to 20 micrograms per cc. during six days of therapy. *E. typhi* could not be cultured from the stools of any of the patients however, after therapy was started.

Two patients with Rocky Mountain spotted fever were treated. The first, an 11-year-old boy, was given 300 mg. every six hours. The temperature began to subside within twenty-four hours and was within normal limits in sixty hours. The rash began to disappear with the drop in temperature and was entirely gone in twenty-four hours. Aureomycin therapy was continued for five days and recovery was uneventful.

The second patient, a 43-year-old man, had been under treatment with sodium para-amino-benzoate for 10 days without effect. He was disoriented and had continuous fever of 104° to 105° F. Other medication was discontinued and aureomycin treatment started in doses of 700 mg. every six hours. Fever and toxicity began to subside within twelve hours. The temperature reached normal, forty-eight hours after the start of therapy, and remained within normal limits thereafter. Aureomycin treatment was given for five days altogether and recovery was uneventful.

Summary

1. Assayable serum concentrations of Aureomycin may be obtained when the antibiotic is given by either the intramuscular or the oral route. The former is somewhat painful so that the oral route is recommended as a routine. Doses at six-hour intervals have been found to be satisfactory, although eight- or twelve-hour intervals may be sufficient.

2. Four patients with typhoid fever showed no clinical or bacteriological response when treated with aureomycin, while two patients with Rocky Mountain spotted fever appeared to recover dramatically after the institution of aureomycin therapy.

Reference

1. Dornbush, A. C., & E. J. Pelcak. 1948. The determination of aureomycin in serum and other body fluids. Ann. N. Y. Acad. Sci. **51**(2): 218–220.

USE OF AUREOMYCIN ON SOME EXPERIMENTAL INFECTIONS IN ANIMALS

By Paul A. Little

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AUREOMYCIN has been tested in mice against experimental infections due to *Eberthella typhosa* and to *Erysipelothrix rhusiopathiae.* Baby chicks have been used to test Aureomycin against experimental infections due to organisms which are responsible for highly fatal, naturally occurring diseases of chicks. These organisms are *Pasteurella multocida* and *Shigella gallinarum.* We have found Aureomycin effective against the infections produced when each of these organisms was inoculated into the experimental animals.

Table 1 summarizes the methods by which the different infecting agents were prepared for animal inoculation.

TABLE I

METHODS OF PREPARING INFECTING AGENTS FOR ANIMAL INOCULATION

ORGANISM	TIME OF INCUBATION OF CULTURE (37° C)	SIZE OF INOCULUM (0.5 ML)
E. TYPHOSA	24 HRS.	SUSPENSION*
E. RHUSIOPATHIAE	16 HRS.	10^{-4} DILUTION
P. MULTOCIDA	6 HRS.	10^{-2} DILUTION
S. GALLINARUM	6 HRS.	10^{-6} DILUTION

*Suspension From Blood Agar Plate Was Standardized To Contain About 1 Billion Organisms Per Dose.

All Dilutions Were Made In 2 Per Cent Peptone Solution.

Eberthella typhosa is of interest as a representative of gram-negative organisms of the typhoid-paratyphoid group. The fact that the organism is not naturally virulent for mice necessitated inoculating suspensions standardized to contain about 1 billion organisms per 0.5 milliliter dose. This amount of culture was obtained by suspending the growth from a blood agar plate in 2 per cent peptone solution. Given intra-abdominally, the dose killed mice in 24 hours.

Erysipelothrix rhusiopathiae is the cause of the economically important disease, swine erysipelas, and is naturally infectious to many animals including man. Our demonstration of the ability of Aureomycin to protect mice against *E. rhusiopathiae* serves to differentiate the mode of action of this antibiotic from that of Polymyxin which is effective against *E. typhosa* but not against *E. rhusiopathiae.* Since the organism is highly virulent for mice, we used 0.5 milliliter of a 10^{-4} dilution of culture grown for 16 hours in bile-enriched yeast-extract broth. The organism has a peculiar nutritional requirement for fatty acids furnished by bile. The inoculum used contained at least 1000 minimum lethal doses and killed mice in 48 to 72 hours.

Pasteurella multocida is of interest as a representative of the group of gram-negative organisms which includes *Pasteurella pestis.* Besides causing fowl cholera, this organism produces rapid and highly fatal infections in all species of domestic and wild animals, but is innocuous to man. We inoculated one-day-old chicks intra-abdominally with 0.5 milliliter of a 10^{-2} dilution of culture grown for 6 hours in yeast-extract broth. This amount of inoculum contained at least 100,000 minimum lethal doses and killed chicks in 24 to 48 hours.

Since it is unusual to employ chicks as laboratory animals for bacteriological studies, it may be of interest that they weigh about 40 grams when one day old and may be injected intra-abdominally as many as six times a day without fatality. We used a pure strain of New Hampshire Red chicks furnished by a local hatchery.

Shigella gallinarum, the cause of fowl typhoid, is of interest because it produces typical symptoms of typhoid in the chick. We used 0.5 milliliter of a 10^{-6} dilution of culture grown for 6 hours in yeast-extract broth. This amount of inoculum, injected intra-abdominally, contained at least 100 minimum lethal doses and killed chicks in 72 to 120 hours. The culture was also virulent when given by mouth.

Experimental Results

In testing Aureomycin against *E. typhosa* in mice, we have studied the effect of starting treatment ½ hour before inoculating the culture, and the effect of delaying treatment until ½ hour after culture. In both experiments the doses of Aureomycin were divided and the half dose was given intra-abdominally at 9 A.M. and 5 P.M. for three days.

Figure 1 shows the endpoint of activity of Aureomycin when treatment was started ½ hour before culture. One milligram per kilogram per day did not protect; two milligrams gave 50 per cent; four milligrams gave 80 per cent; eight milligrams gave 100 per cent protection. The actual amounts of Aureomycin administered may be of interest. To obtain 50 per cent protection, doses of 0.02 milligram were given at 9 A.M. and 5 P.M. for three days. To obtain 100 per cent protection, doses of 0.08 milligram were given.

Figure 2 shows the effect of delaying treatment until ½ hour after inoculating the culture. The range of from 5 to 40 milligrams per kilogram per day includes the smallest amount of Aureomycin expected to give complete protection on the basis of the results of the previous ex-

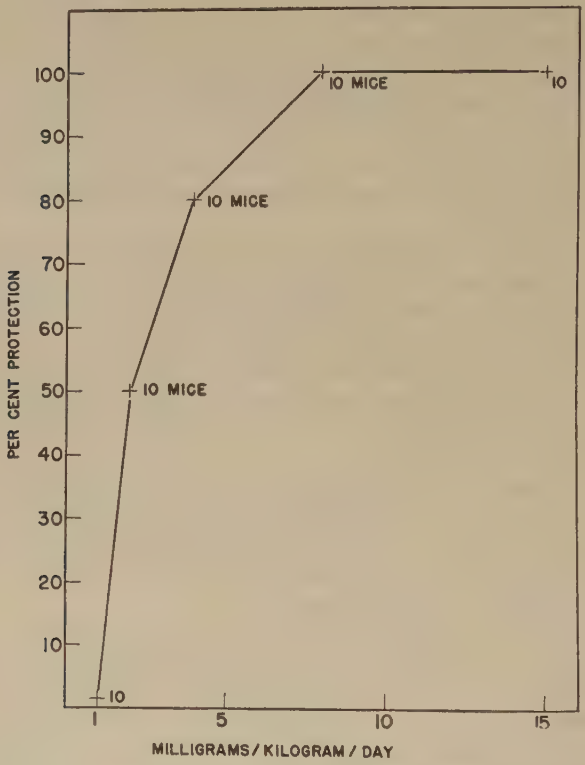

FIGURE 1. Action of Aureomycin against *E. typhosa.* Treatment started ½ hour before culture.

periment. This amount of Aureomycin gave 90 per cent protection under the conditions of the test. To obtain this result doses of 0.05 milligram were given at 9 A.M. and 5 P.M. for three days. Only 70 and 80 per cent protection were obtained with 10 and 20 milligrams per kilogram. Complete protection was obtained with 40 milligrams. The curve does not show a different endpoint of activity from that demonstrated in our first experiment. Since 100 per cent of mice receiving 40 milligrams per kilogram lived, the curve does not show a loss of mice which might be attributed to toxicity of the antibiotic. The losses of mice incurred in this experiment appear to be due solely to the severity of the test conditions. In other words, the results illustrate the well-known importance of early treatment.

FIGURE 3 shows the endpoint of activity of Aureomycin when used to treat mice infected with *E. rhusiopathiae.* The doses were divided and the half dose given at 9 A.M. and 5 P.M. for three days, starting ½ hour before culture. Four milligrams per kilogram per day did not protect; eight milligrams gave 20 per cent; fifteen milligrams gave 80 per cent; thirty milligrams gave complete protection. To obtain 50 per cent protec-

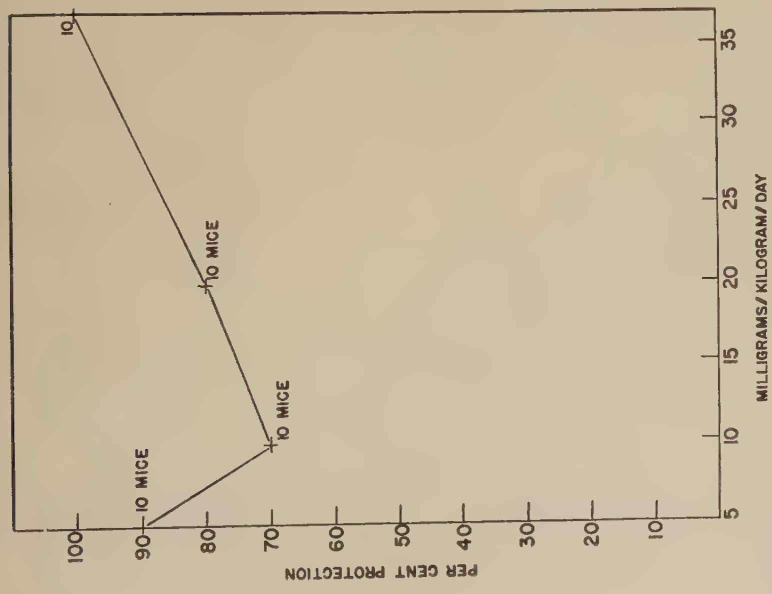

FIGURE 2. Action of Aureomycin against *E. typhosa.* Treatment started ½ hour after culture.

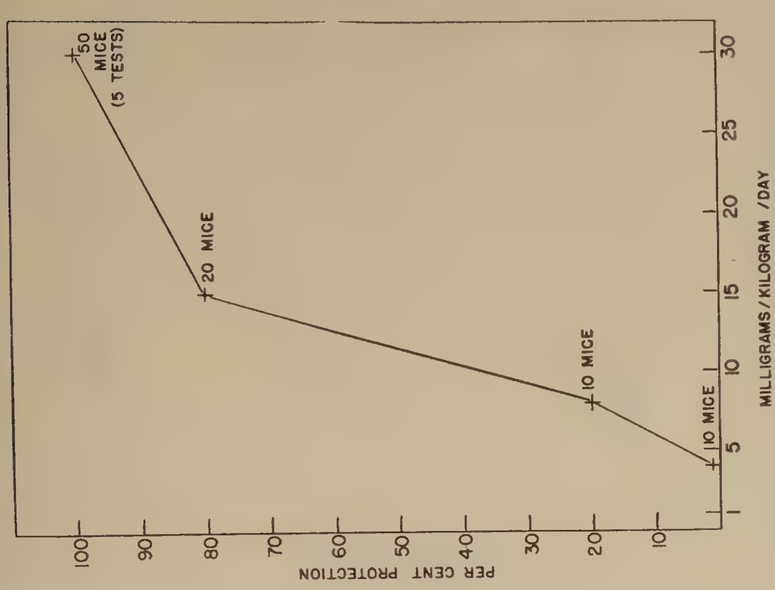

FIGURE 3. Action of Aureomycin against *E. rhusiopathiae.* Treatment started ½ hour before culture.

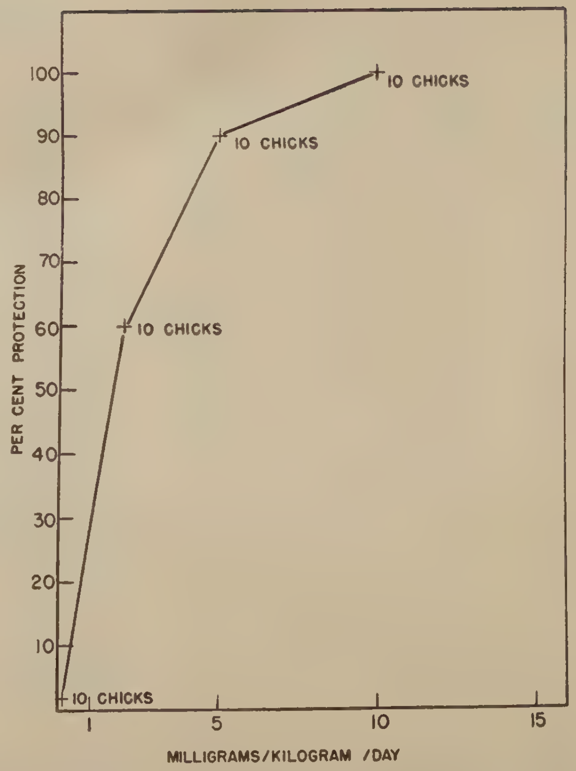

FIGURE 4. Action of Aureomycin against *P. multocida*. Treatment started ½ hour before culture.

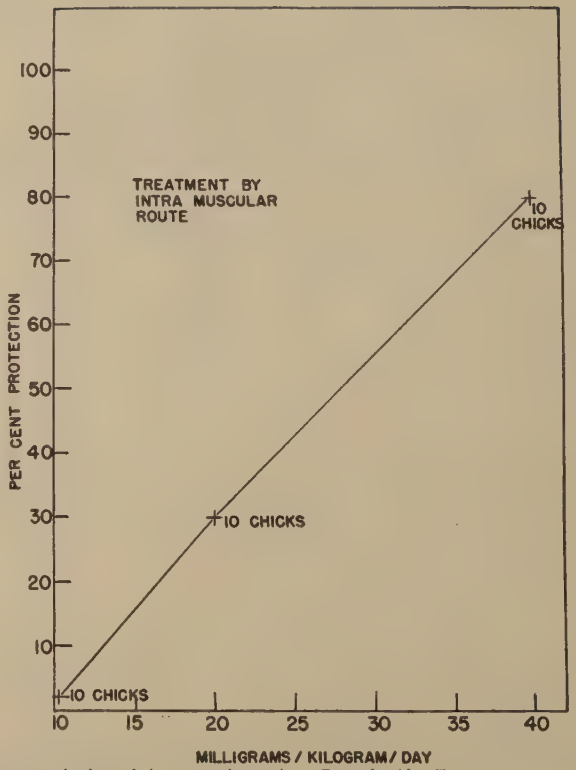

FIGURE 5. Action of Aureomycin against *P. multocida*. Treatment started ½ hour after culture.

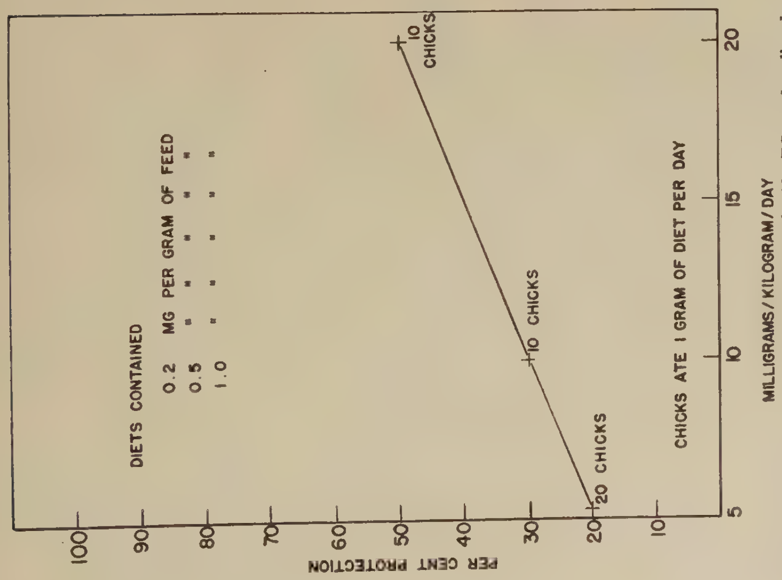

FIGURE 6. Action of Aureomycin against *P. multocida*. Effect of orally administered Aureomycin.

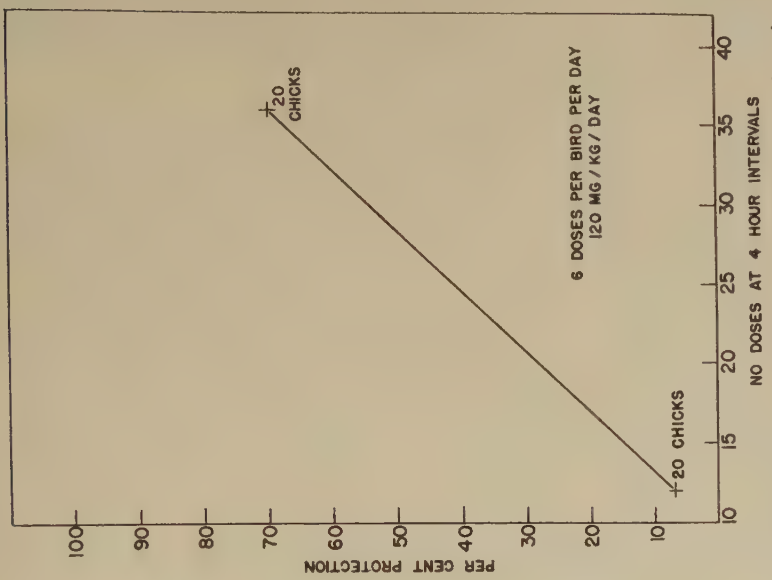

FIGURE 7. Action of Aureomycin against *S. gallinarum*. Effect of treating at 4 hour intervals using doses of 1 mg.

tion would require 11.5 milligrams as compared to 2 milligrams required in our tests with *E. typhosa*. Apparently 5 to 6 times as much Aureomycin is required in treating infections due to *E. rhusiopathiae* as in treating infections due to gram-negative bacteria. It is of interest that penicillin is also effective against *E. rhusiopathiae.*

FIGURE 4 shows the endpoint of activity of Aureomycin when used to treat one-day old chicks infected with *P. multocida*. In this experiment, the chicks received intra-abdominal injections of Aureomycin once daily for four days, starting ½ hour before culture. Two milligrams per kilogram gave 60 per cent protection; five milligrams gave 90 per cent; and ten milligrams gave complete protection. Even though the chicks received only one treatment per day, the amount of Aureomycin required to give 50 per cent protection corresponds closely with the result of our tests with *E. typhosa*. In these experiments, two milligrams per kilogram per day appears to be the smallest effect dosage of Aureomycin.

FIGURE 5 shows the results of an experiment in which Aureomycin was administered by the intramuscular route and treatment was delayed until ½ hour after culture. The doses were divided and the half dose given in the right leg at 9 A.M. and 5 P.M. for four days. Ten milligrams per kilogram failed to protect; twenty milligrams gave 30 per cent protection; and forty milligrams gave 80 per cent protection against *P. multocida*. This experiment answers a question which might be raised with respect to the previous experiments as to whether or not the antibiotic is active when given by a different route from that used in inoculating the culture.

FIGURE 6 shows the effect of orally administered Aureomycin in treating chicks infected with *P. multocida*. The antibiotic was mixed with a commercial chick starter and fed *ad libitum* for four days, starting 24 hours before culture. The chicks were one day old when introduced to the diet and two days old when inoculated with culture. At this age, chicks eat about 1 gram of feed per day. This approximation was used in determining the position of the curve with respect to milligrams of antibiotic per kilogram body weight per day. Three preparations of feed were used in this experiment. One contained 0.2 milligram of Aureomycin per gram; one contained 0.5 milligram; and one contained 1 milligram. In preparing 1 milligram of Aureomycin per gram of feed, we dissolved 500 milligrams of antibiotic in 5 milliliters of distilled water and added this to 500 grams of commercial chick starter. The container was cleaned by the absorbing action of the dry feed.

The diet containing the smallest amount of antibiotic had some protective effect since the culture regularly killed 100 per cent of untreated controls. The diet containing 1 milligram of antibiotic per gram protected 50 per cent of chicks. These chicks appear to have consumed 20 milligrams of antibiotic per kilogram body weight per day.

FIGURE 7 shows the action of Aureomycin against *S. gallinarum* in chicks. In preliminary experiments we had found that this organism was resistant to twice daily doses of the antibiotic. In FIGURE 7 we have compared the effect of 12 doses of 1 milligram, given at 4-hour intervals, and the effect of 36 doses. The more intensive treatment protected 70 per cent of chicks.

TREATMENT OF EXPERIMENTAL INFECTIONS WITH AUREOMYCIN[*][†]

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AUREOMYCIN is an antibiotic derived from a strain of *Streptomyces*. It was supplied as a yellow crystalline hydrochloride salt which is soluble in distilled water but somewhat less soluble in normal saline solution. These solutions are acid (pH 4.5) and their activity deteriorates rapidly with increasing pH at room temperature.

Much of the *in vitro* work, including the methods of biological assay utilized in securing body fluid levels, has been described by Dr. Chandler and Dr. Bliss.[‡] It is the purpose of this presentation to describe some of the pharmacological studies and experimental infections which have been performed.

Toxicity. The acute toxicity of aureomycin was determined in mice and dogs. All mice survived a single intravenous injection of 50 mg./kg. Six out of 7 mice died after receiving 100 mg./kg. Dogs developed transient hyperpnea, generalized weakness, and anorexia following rapid intravenous injection of 50 and 100 mg./kg. Injection of 150 mg./kg. was followed by grunting respirations, tremors, generalized paresis, and somnolence. Death occurred 6 hours after administration of this dose. At autopsy, hemoglobinuria was noted in the bladder urine. Since all intravenous injections were with 10 per cent aureomycin in distilled water, the acidity of such a large dose may well have caused hemolysis.

The majority of mice survived, for a period of 7 days, single subcutaneous injections up to 3000 mg./kg. Only occasional deaths occurred in animals receiving 1000 to 3000 mg./kg. All mice died when 4000 mg./kg. was administered subcutaneously. Death was preceded by hyperpnea, anorexia, tremors, ataxia, and paresis.

Rats tolerated subcutaneous injections of 50 mg./kg. once daily for 8 days with only slight weight loss and mild local inflammatory reaction at the injection site. A dog which received 20 mg./kg. intramuscularly twice a day for 9 days had marked persistent anorexia and progressive weight loss. Induration, necrosis, and fluctuation of the injected tissues was noted. The rats and dogs were injected with aureomycin at a concentra-

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[*] Aureomycin used in these experiments was supplied by the Lederle Laboratories Division, American Cyanamid Company.

[†] These investigations were supported by grants received from Abbott Laboratories; Eli Lilly and Company; Lederle Laboratories Division, American Cyanamid Company; Parke, Davis and Company; and the Upjohn Company.

[‡] See their paper in this monograph.

TABLE 1

ACUTE TOXICITY OF AUREOMYCIN (HCL)

Mode of administration	*Dose mg./kg.*	*Animal*		*Death in 2 days*	*Survival 7 days*
		Type	*No.*		
Intravenous (rapid)	50	Mice	3	0	100%
	100	Mice	7	6	14%
	250	Mice	1	1	0
	500	Mice	1	1	0
	50	Dog	1	0	1
	100	Dog	1	0	1
	150	Dog	1	1	0
Subcutaneous	500	Mice	10	0	100%
	1000	Mice	14	1	93%
	1500	Mice	10	1	90%
	2000	Mice	17	2	88%
	3000	Mice	6	2	77%
	4000	Mice	5	5	0

Signs of intoxication—Rapid respirations, tremors, ataxia, and paresis.

tion of 2.0 to 10.0 per cent in 1.0 per cent procaine hydrochloride in distilled water.

Three drops of 0.25 per cent and 1.0 per cent aureomycin borate solution were instilled locally into the conjunctival sac of a rabbit. No immediate or delayed local reaction was noted.

TABLE 2

AUREOMYCIN (HCL) CHRONIC TOXICITY

Animal	*Dosage schedule*			*Total dose mg.*	*Observed for 10 days Remarks**
	Daily dose mg./k.	*No. doses per day*	*No. days administered*		
Rat 1	50 S.C.	1	8	112	12 gm. wt. loss
2	50 S.C.	1	8	130	10 gm. wt. loss
3	Control	–	–	—	3 gm. wt. gain
4	Control	–	–	—	20 gm. wt. gain
Dog 1	40 I.M.	2	9	2,280	1200 gm. wt. loss. Marked reaction at site of injections
2	40 I.M.	1	1	160	Well tolerated
Rabbit 1	20 I.M.	1	1	70	Well tolerated
2	Locally in the eye (borate) 0.25%	1	1	0.5	No reaction
	1.0%	1	1	2.0	No reaction

S.C. = Subcutaneously.
I.M. = Intramuscularly.
Locally—3 drops of the *borate* was instilled locally in the eye.
* Animals were observed for changes in weight and hemoglobin. Urine was examined for proteinuria, volume, specific gravity, and microscopic elements.

TABLE 3

ASSAY OF BODY FLUIDS OF ANIMALS INJECTED INTRAMUSCULARLY WITH AUREOMYCIN

Animal	*Dosage schedule*	*Day of administration*	*Interval post last injection*	*Levels of aureomycin gamma/cc.*		
				Serum	*Sp. fl.*	*urine*
Rabbit	20 mg./kg. single dose	1	0	0		
			30 Min.	1.25		
			1 Hour	1.25		
			2.5 Hrs.	0		
			3.5 Hours	0		
			6 Hours	0		
Dog 1	40 mg./kg. single dose	1	0	0	0	
			15 Min.	1.2	0	
			30 Min.	Trace	0	
			1 Hour	0	0	
			2 Hours	0	0	
2	20 mg./kg. B.I.D. for 10 days	1	0	0		
			1 Hour	Trace		
		2	1 Hour	0		
		4	15 Hours	0		
			1 Hour	1.25		
		6	15 Hours	Trace		
			10 Hours	Trace		
		10	5 Min.	0.6	0	
			30 Min.	0.6	0	
			2.5 Hours	0.3	0	>64

Autopsies were performed on mice, rats, and dogs which had received large (up to 3000 mg./kg.) and repeated doses (total up to 400 mg./kg. over a period of 9 days) of aureomycin. No gross or microscopic* abnormalities in the liver, kidney, heart, or bone marrow were noted in any of the animals. However, necrosis of tissue occurred at the local injection sites. Mice which had received aureomycin by intubation showed evidence of local erosions of the gastric mucosa. These were colored yellow by the drug.

Levels in Body Fluids. Blood serum levels were obtained following single intramuscular injections of 20 mg./kg. in rabbits and 40 mg./kg. in dogs. Levels of 1.25 micrograms/ml. were recorded in the serum 15 minutes to one hour following the injections. No significant serum concentrations could be measured when serum was obtained more than one hour after injection of the drug. A dog received 20 mg./kg. twice a day for 10 days. No significant level was determined in the serum obtained more than 2.5 hours after the last injection. Blood drawn at regular intervals one hour after injection during the period of administration of aureomycin contained 0.3 to 1.25 micrograms/ml. of serum. A random specimen of urine obtained on the 10th day contained 64 micrograms of aureomycin per ml. The antibiotic was not detected in the spinal fluid after parenteral administration.

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* Microscopic sections were prepared and their interpretation corroborated by Dr. Tobias Weinberg, Pathologist, Sinai Hospital, Baltimore, Maryland.

Therapeutic Effectiveness: Blood Cultures. Mice were infected intraperitoneally with 10,000 LD's of *Pneumococcus* type I (SVI) and *Streptococcus hemolyticus* beta (C203). Complete clearing of the blood stream infection occurred when a loopful of tail blood was cultured 18 hours after subcutaneous treatment with three injections of aureomycin or penicillin G 5 mg./kg. each. The control mice all died with positive blood cultures in this period. Penicillin G protected a higher proportion of the mice from death when observed over a 7 day period. Only partial clearing of the *K. pneumoniae* A from the bloodstream was obtained with a dosage schedule of 10 mg./kg. three times daily. Polymyxin gave complete clearing of the bacteremia, and streptomycin almost complete clearing. The dosage of polymyxin and streptomycin employed was only one-tenth that of aureomycin used above. None of the mice which had received aureomycin survived a 7-day period, while 40 per cent survived when treated with the smaller dosages of polymyxin or streptomycin.

Table 4

Blood Cultures in Mice* Treated With Aureomycin, Polymyxin, or Streptomycin

(Hemolytic streptococcus C203, *Pneumococcus* SVI, or *K. pneumoniae* infection)

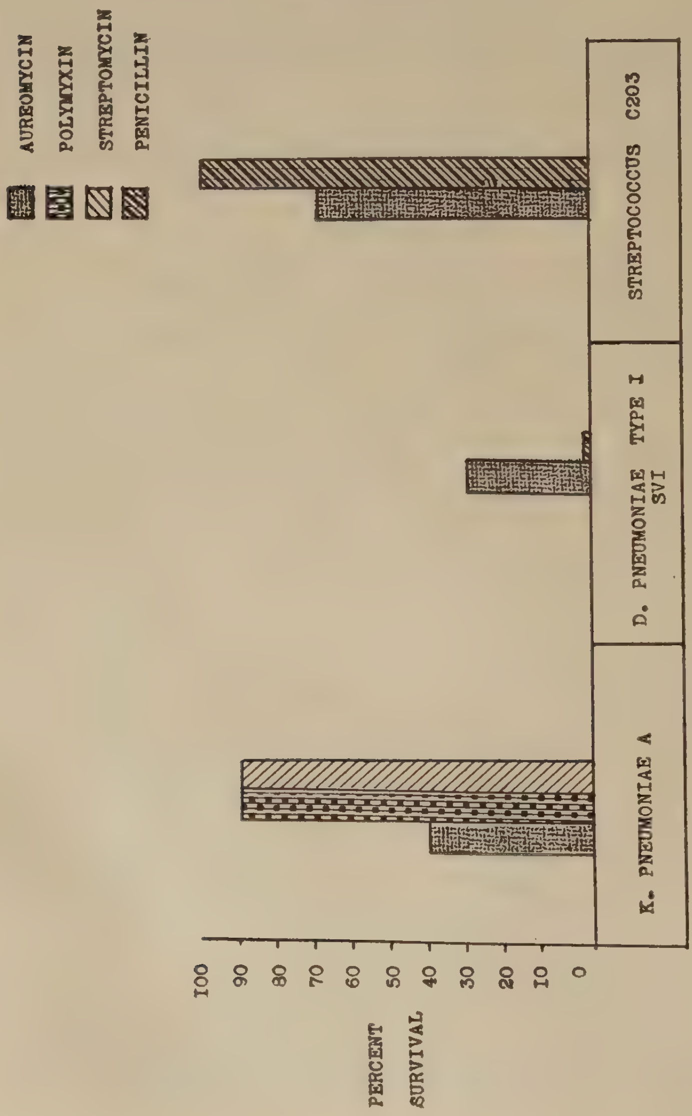
No. mice	*Organism*	*Drug*	*Dose mg./kg.*	*Time after infection*	*Tail blood cultures Mouse*					*Per cent survival 7 days*
					1	2	3	4	5	
	K. pneumoniae 10,000 LD'S	Untreated mice	0	2 Hrs.	+	+	+	+	+	
10		Aureomycin	30	20 Hrs.	0	+	0	+†	+†	0
10		Polymyxin	3		0	0	0	0	0	40
10		Streptomycin	3		0	0	+	0	0	40
16		Control	0		+†	+†	+†	+†	+†	0
	Hemolytic streptococcus C203 10,000 LD'S	Untreated mice	0	2 Hrs.	0	0	0	0	+	
10		Aureomycin	15		0	0	0	0	0	50
10		Penicillin G	15	20 Hrs.	0	0	0	0	0	80
16		Control	0		+†	+†	+†	+†	+†	0
	Type I *Pneumococcus* SVI 10,000 LD'S	Untreated mice	0	2 Hrs.	+	+	+	+	+	
10		Aureomycin	15	20 Hrs.	0	0	0	0	0	90
10		Penicillin G	15		0	0	0	0	0	100
16		Control	0		+†	+†	+†	+†	+†	0

* Albino Swiss mice 18–22 gm. Initial dose of drug 2 hours post intraperitoneal infection and repeated T.I.D. for 3 days. Treatment subcutaneously.

† Dead at 20 hours post infection.

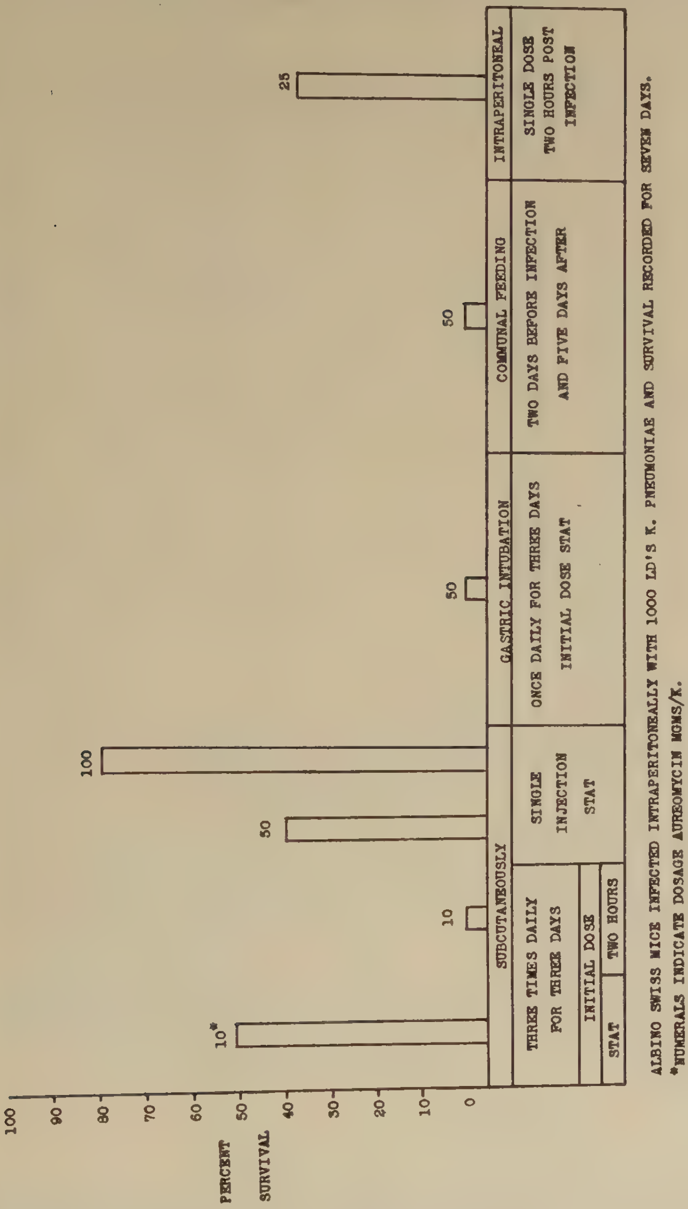
Subcutaneous Injection. A single subcutaneous injection of 50 mg./kg. of aureomycin administered immediately after infection, protected 40 per cent of mice infected intraperitoneally with 1000 LD's of *K. pneu-*

moniae A, 30 per cent of those infected with *Pneumococcus* type I (SVI), and 60 per cent of *Streptococcus hemolyticus* beta (C203) infected mice. Both polymyxin and streptomycin at the same dosage resulted in 90 per cent of mice surviving the *K. pneumoniae* infection for the 7-day observation period. A *single* injection of aureomycin was more effective than penicillin G when mice were infected with *Pneumococcus* type I (SVI). It was less effective than penicillin G when *Streptococcus hemolyticus* beta (C203) was the infecting organism.



*ALBINO SWISS MICE INFECTED INTRAPERITONEALLY WITH 1000 LD'S OF K. PNEUMONIAE A, PNEUMOCOCCUS SVI, OR STREPTOCOCCUS C203 AND TREATED IMMEDIATELY. SURVIVAL RECORDED FOR 7 DAYS. (10 MICE IN EACH GROUP)

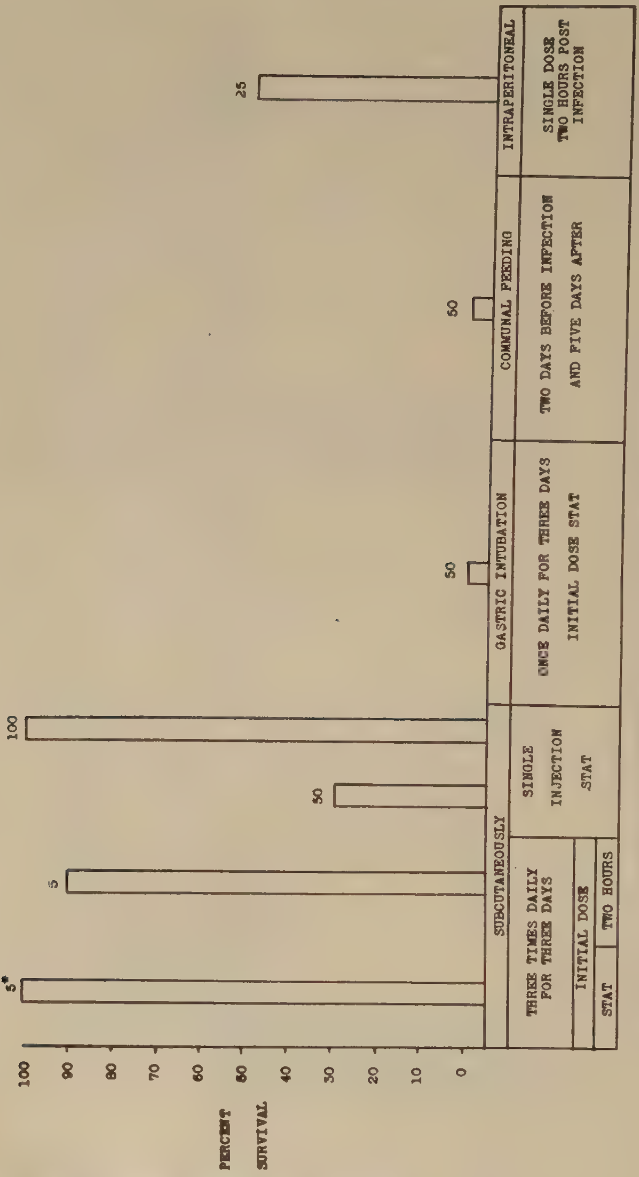
FIGURE 1. Comparison of a single subcutaneous dose (50 mg./kg.) of aureomycin, polymyxin, streptomycin, and penicillin.*



ALBINO SWISS MICE INFECTED INTRAPERITONEALLY WITH 1000 LD'S K. PNEUMONIAE AND SURVIVAL RECORDED FOR SEVEN DAYS.
*NUMERALS INDICATE DOSAGE AUREOMYCIN MGMS/K.
(10 MICE IN EACH GROUP)

FIGURE 2. Comparison of different modes of administration of aureomycin, *K. pneumoniae* type A infection.

Modes of Administration. The protection of mice infected intraperitoneally with 1000 lethal doses of *K. pneumoniae* A, *D. pneumoniae* type I (SVI), and *Streptococcus hemolyticus* beta (C203), when treated with aureomycin administered by various routes, was investigated. Com-



ALBINO SWISS MICE INFECTED INTRAPERITONEALLY WITH 1000 LD'S OF D. PNEUMONIAE TYPE I (SVI) AND SURVIVAL RECORDED FOR SEVEN DAYS.

*NUMERALS INDICATE DOSAGE AUREOMYCIN MGMS/K.

(10 MICE IN EACH GROUP)

FIGURE 3. Comparison of different modes of administration of aureomycin, *D. pneumoniae* type I (SVI) infection.

munal feeding was begun 2 days before infection and continued for 5 days thereafter, so that a daily dose of approximately 50 mg./kg. was ingested. The only survivors observed at the end of 7 days were those mice infected with *Streptococcus hemolyticus* beta (C203). Seventy per cent protection was noted. Similarly, gastric intubation with 50 mg./kg. begun immediately after infection and continued once each day for 3 days

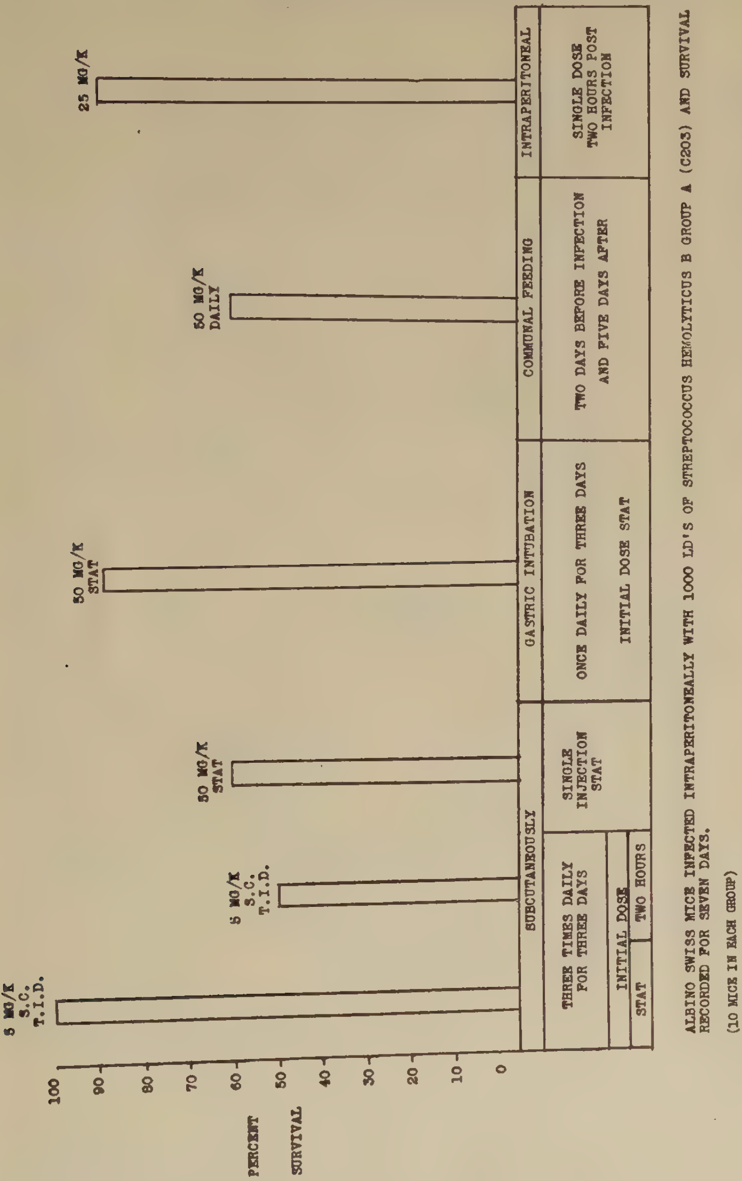
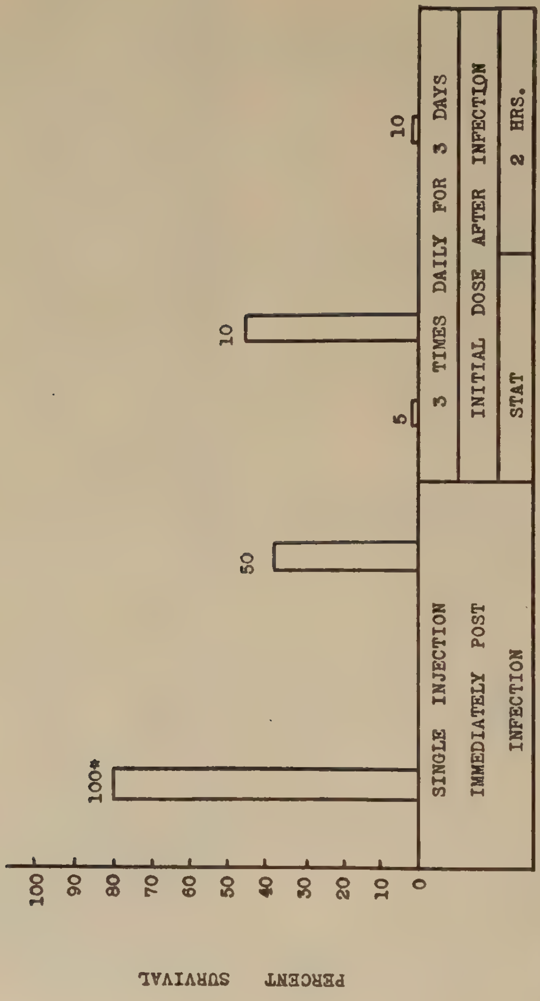


FIGURE 4. Comparison of different modes of administration of aureomycin, *Streptococcus hemolyticus* B group A (C203) infection.

protected 90 per cent of mice infected with *Streptococcus hemolyticus* only. Subcutaneous administration of aureomycin gave some protection against all organisms both when a single or multiple dose schedule was employed. Intraperitoneal injection was more effective than the subcutaneous route, although the former therapy was not instituted until 2 hours after infection.



ALBINO SWISS MICE INFECTED INTRAPERITONEALLY WITH 1000 LDS K. PNEUMONIAE AND SURVIVAL RECORDED FOR 7 DAYS.

* NUMERALS INDICATE DOSAGE AUREOMYCIN MGMS/K S.C.
(10 MICE IN EACH GROUP)

FIGURE 5. Comparison of single and multiple dose schedules in mice treated subcutaneously with aureomycin (*K. pneumoniae* infection).

Single and Multiple Doses. Comparison of different dosage schedules administered subcutaneously to mice infected with 1000 LD's of *K. pneumoniae* A, *D. pneumoniae* type I, and *Streptococcus hemolyticus* beta, revealed that 5 to 10 mg./kg. given 3 times daily for 3 days resulted in more survivors than 50 mg./kg. given as a single dose. In the *Pneumococcus* infection, 5 mg./kg. 3 times a day for 3 days protected as well as a single injection of 100 mg./kg. A 2 hour delay in treatment following infection resulted in a reduction of survivors.

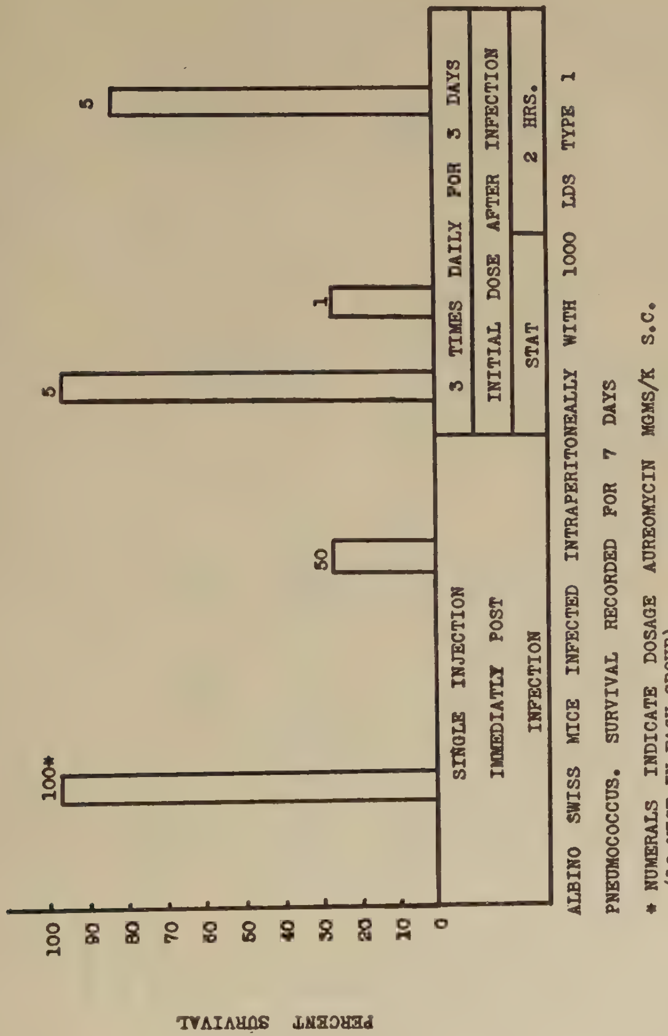


FIGURE 6. Comparison of single and multiple dose schedule in mice treated subcutaneously with aureomycin, type 1 *Pneumococcus* infection.

Comparison of Aureomycin, Penicillin, Polymyxin, and Streptomycin. Parallel experiments were performed in mice infected with the organisms mentioned above. Penicillin G was employed for the infections with the gram positive organisms, while streptomycin and polymyxin were used for the *K. pneumoniae* type A infection. Aureomycin, when compared on

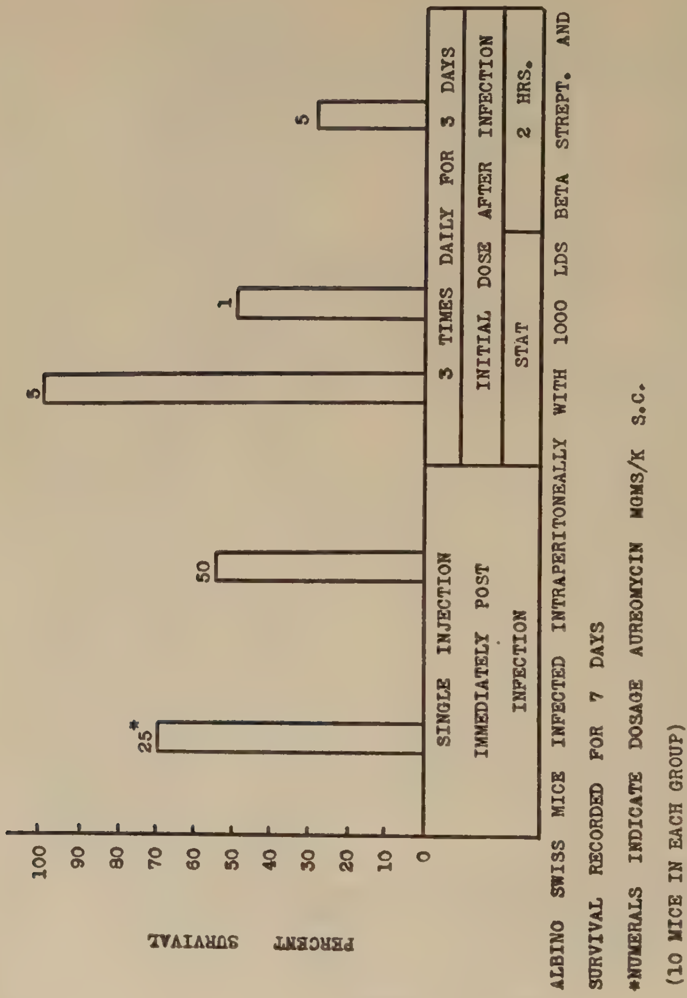


FIGURE 7. Comparison of single and multiple dose schedules in mice treated subcutaneously with aureomycin, beta hemolytic streptococcus infection.

a mg./kg. basis, was as effective as penicillin when *Pneumococcus* type I was the infecting organism. Protection achieved against *Streptococcus hemolyticus* infection was somewhat inferior to penicillin. When all routes of administration were compared, the protection of mice infected with *K. pneumoniae* A and treated with aureomycin was definitely below that observed with polymyxin and streptomycin.

TABLE 5

COMPARISON OF AUREOMYCIN, POLYMYXIN AND STREPTOMYCIN (*K. Pneumoniae* INFECTION)*

	Dosage schedules				*Per cent survival 7 days post infection*			
Mode of administration	*Hours after infection*	*Injections per day*	*Daily dose mg./kg.*	*Total days treated*	*Aureomycin*	*Polymyxin*	*Streptomycin*	*Controls*
Subcutaneous	0	1	100.0	1	80			0
			5.0			60		0
			50.0		40	90	90	0
		3	15.0	3	0	100		0
			30.0		50			0
			1.5			60	20	0
	2	2	30.0	3	0			
			3.0			40	40	0
Intraperitoneal	2	1	25.0	1	40	60	80	0
Gastric intubation	0	1	50.0	3	0	70	50	0
Communal feeding	2 Days before infection	Fed continuously	50 circa	7	0	0	0	0

* Albino Swiss mice infected intraperitoneally with 1000 LD's of *K. pneumoniae* A (10 mice in each group).

TABLE 6

COMPARISON OF AUREOMYCIN AND PENICILLIN (*Pneumococcus* SVI INFECTION)*

	Dosage schedule				*Per cent survival 7 days post infection*		
Mode of administration	*Hours after infection*	*Injections per day*	*Daily dose mg./kg.*	*Total days treated*	*Aureomycin*	*Penicillin*	*Controls*
Subcutaneous	0	1	100.0	1	100	20	0
			50.0		30	0	0
		3	15.0	3	100	100	0
			3.0		30	90	0
	2	3	15.0	3	90	100	0
Intraperitoneal	2	1	25.0	1	50	0	0
Gastric intubation	0	1	50.0	3	0	0	0
Communal feeding	2 Days before infection	Fed continuously	50 circa	7	0	0	0

* 10 Albino Swiss mice infected intraperitoneally with 1000 LD's of Type I *Pneumococcus* (SVI) in each group.

Table 7

Comparison of Aureomycin and Penicillin (*Streptococcus* C302 Infection)*

Mode of administration	*Dosage schedule*				*Per cent survival 7 days post infection*		
	Hours after infection	*Injections per day*	*Daily dose mg./kg.*	*Total days treated*	*Aureomycin*	*Penicillin*	*Controls*
Subcutaneous	0	1	25.0	1	70	90	10
			50.0		80	100	0
		3	15.0	3	100	100	0
			3.0		30	90	0
	2	3	15.0	3	50	80	0
Intraperitoneal	2	1	25.0	1	90	90	0
Gastric intubation	0	1	50.0	3	90	60	0
Communal feeding	2 Days before infection	Fed continuously	50 circa	7	70	90	0

* Albino Swiss mice infected intraperitoneally with 1000 LD's of group A beta hemolytic streptococcus C203 (10 mice in each group).

Summary

1. The approximate LD_{50} for aureomycin administered intravenously to mice is between 50 and 100 mg./kg. The LD_{50} for subcutaneous injection is between 3000 and 4000 mg./kg. Rapid intravenous injection of 150 mg./kg. resulted in hemoglobinuria and death of a dog.

2. Repeated subcutaneous and intramuscular doses of aureomycin were well tolerated in rats and dogs except for local irritation, anorexia and weight loss. Autopsies revealed no gross or microscopic abnormalities of the viscera.

3. A rabbit tolerated 1.0 per cent aureomycin borate locally in the eye.

4. Serum levels of 0.3 to 1.25 micrograms/ml. were obtained within 1 hour after parenteral administration. No antibiotic was detected in the spinal fluid. Urine contained a high concentration of aureomycin.

5. Aureomycin administered to mice orally did not protect against *Pneumococcus* type I or *K. pneumoniae* A infection, but did protect against *Streptococcus hemolyticus* beta. Parenteral doses gave some protection against all three infections. Multiple injections resulted in more survivors than the same amount given in a single dose. Delay in treatment resulted in a reduction of survivors. Penicillin afforded slightly more protection against infection with the gram positive cocci. Polymycin and streptomycin were superior in the *K. pneumoniae* infections.

THE PHARMACOLOGY AND CLINICAL TRIAL OF AUREOMYCIN: A PRELIMINARY REPORT[*][†]

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AUREOMYCIN (A-377) is an antibiotic derived from a strain of *Streptomyces. In vitro* it has been shown to possess bacteriostatic and bactericidal activity *versus* various gram positive and gram negative bacteria. Protection of laboratory animals experimentally infected with a variety of bacterial and rickettsial agents as well as the virus of lymphopathia venereum has been demonstrated. The toxicity of this antibiotic in mice and dogs was relatively low and clinical trial in selected infections was undertaken.

The drug was obtained from the Lederle Laboratories Division of the American Cyanamid Company. It was supplied as the crystalline hydrochloride salt in sterile vacuum dried ampoules each containing 20 milligrams of drug (Lot numbers 7-7845, 7-8071A and 7-8071B), and more recently in gelatin capsules each containing 50 milligrams (Lot number 7-8378). The antibiotic is a yellow powder soluble in distilled water and 5 per cent glucose in distilled water. Solutions in normal saline precipitate when a concentration greater than 1 per cent of the drug is attained. The aqueous solutions are acid and when neutralized or made alkaline deterioration of activity is rapid at room temperature.

When the drug was injected intramuscularly into patients, dissolved in distilled water (also 2.5 and 5.0 per cent glucose solution), acute pain lasting several minutes followed by a dull drawing pain which persisted for approximately one-half hour was noted. The highest concentration of aureomycin attempted was a 1 per cent solution (20 milligrams in a volume of 2.0 cc.). Upon repeated dosages the local sites became erythematous and tender. When the drug was dissolved in 1 per cent novocaine, 40 milligrams could be injected in a volume of 2.0 cc. The acute pain following the injection was then not observed, but the dull drawing pain did appear. Treatment of patients *via* the intramuscular route did not appear feasible in light of the low local tolerance to the drug.

Oral dosage of aureomycin was well tolerated. A single dose of 0.5 grams of a crude preparation was associated with loss of appetite and nausea. With more purified drug in gelatin capsules, nausea and vomiting have been noted in only one patient on repeated occasions. The nausea in this patient was maximal following the early morning doses and was completely relieved when ½ ounce of an aluminum hydroxide preparation (amphojel) was given with each 100 milligram dose of drug. Other patients and normal human subjects have exhibited no nausea on oral dosage of aureomycin. The highest dosage schedule employed to date has been 100 milligrams every two hours for a patient who weighed 40 kilograms.

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[*] Aureomycin used in these experiments was supplied by the Lederle Laboratories Division, American Cyanamid Company.

[†] These investigations were supported by grants received from Abbott Laboratories; Eli Lilly and Company; Lederle Laboratories Division, American Cyanamid Company; Parke, Davis and Company; and the Upjohn Company.

Attempts to determine blood levels of this antibiotic have been quite disappointing. One hour following an oral dose of 0.5 grams and an intramuscular injection of 40 milligrams, a level of 0.6 micrograms per milliliter of serum was obtained. When the drug had been given for 5 to 14 days, intramuscular injection of 40 milligrams was followed by serum levels of 1.2 to 2.4 micrograms per milliliter. On oral dosage alone, no detectable blood levels have been obtained. The determination is beset with many difficulties, among which may be noted the neutralizing effect of serum proteins, the deterioration of the drug in alkaline solution, and the relatively high concentration of the antibiotic necessary for minimal inhibition of growth of the test organism, *K. pneumoniae* type A. A level of 0.6 micrograms per milliliter of serum is the lowest concentration of drug that can be measured with the present procedure.* We are trying to improve these assay methods for blood. Early trials to devise a chemical method employing the characteristic ultraviolet absorption spectrum plus extraction with organic solvents have been unsuccessful.

The urinary concentration of aureomycin after intramuscular or oral administration has been quite high. A slight delay of six to twelve hours after oral administration has been noted before urinary excretion in adequate concentration has been observed. Thence, on an oral dosage schedule of 15 mg./kg. per day urine levels of 20-80 micrograms per milliliter were obtained. When the oral dosage was 30 mg./kg. per day the urinary concentration of aureomycin was 80-160 micrograms per milliliter.

Patients and normal individuals have been given aureomycin for periods of five to twenty days on a dosage schedule of 15-30 mg./kg. per day. The age range of these patients was three years to forty-five years. Aside from the nausea noted with oral dosage in two patients and the local irritation following intramuscular administration, no evidence of toxicity has been noted. There has been no evidence of urinary impairment as measured by volume, specific gravity, albuminuria, microscopic examination, blood non-protein nitrogen levels or excretion of phenolsulphonphthalein. No jaundice or change in icteric index, cephalin flocculation or prothrombin has been noted. Repeated hematological examination of the peripheral blood has not indicated any evidence of anemia, hemolytic process, leucopenia, or depression of platelets. Chemical examinations of the blood have not indicated any deviation in total protein, albumin-globulin content or ratio, cholesterol, carbon-dioxide, or alkaline phosphatase values. No idiosyncratic type of response such as rash, hives, drug fever, vertigo, vasomotor reactions, or polyneuritic phenomena have been encountered to date in this comparatively small series.

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* Assays of blood and urine for aureomycin were conducted by Dr. Caroline A. Chandler, Department of Preventive Medicine, Johns Hopkins University, School of Medicine.

Some of the cases treated may serve to illustrate our clinical experience.

The first patient (FIGURE 1) is a 15-year-old girl who has suffered from a chronic pyelonephritis since the age of six. Therapy with sulfonamide drugs, penicillin and streptomycin had been employed singly and in combination with temporary clinical response, but since September, 1947, a continued low grade fever and pyuria were present. Repeated exacerbations of fever up to 106°, and chills had occurred. Pleurisy with effusion, jaundice, and azotemia had occurred in association with these febrile episodes. A biopsy in September 1947 of muscle and artery showed no evidence of vascular collagen disease and an exploratory abdominal laparotomy in February 1948 revealed no abnormality except for an enlarged right kidney. As all sulfonamide and antibiotic therapy had been associated with incomplete and only temporary amelioration of symptoms, she was transferred early in May, 1948, from a hospital in Florida to the Johns Hopkins Hospital for further investigation and therapy.

Examination of the urinary tract with retrograde pyelography revealed dilatation of both ureters below the uretero-pelvic junction. The calyces were somewhat dilated on the right side. A heavy growth of *coli-aerogenes* was obtained from both ureteral specimens. Repeated urine examinations were consistently abnormal with albuminuria, white blood cells in clumps, and microscopic pyurea. Urine cultures were persistently positive for *coli-aerogenes* and, at various times, *Streptococcus hemolyticus* β and enterococci also were recovered. Numerous examinations for acid-fast bacilli on smear or culture of the urine were negative. The coliform organism was resistant to 250 micrograms per milliliter of streptomycin and penicillin *in vitro*. The enterococcus was found to be sensitive to 0.9 micrograms of penicillin, but resistant to 250.0 micrograms of streptomycin per milliliter.

An attempt to sterilize the urine was made by giving 1.2 million units of penicillin G, and 2.4 grams of streptomycin intramuscularly daily. In addition, 6.0 grams of sulfadiazine and 10–12 grams of sulfasuxidine were given orally each day. While on this regime, her temperature fell to 99°-100° F., but pyuria and positive urine cultures persisted. This multiple drug treatment was continued for eighteen days. Her temperature rose to 104.6° F. twenty-four hours after the antibiotic and sulfadiazine were discontinued.

Trial of aureomycin was then considered. This antibiotic is excreted in the urine and high concentrations can be obtained. The *coli-aerogenes* organism was inhibited by 5.0 micrograms per milliliter *in vitro*. Oral aureomycin in a daily dose of 600 milligrams divided into six doses was

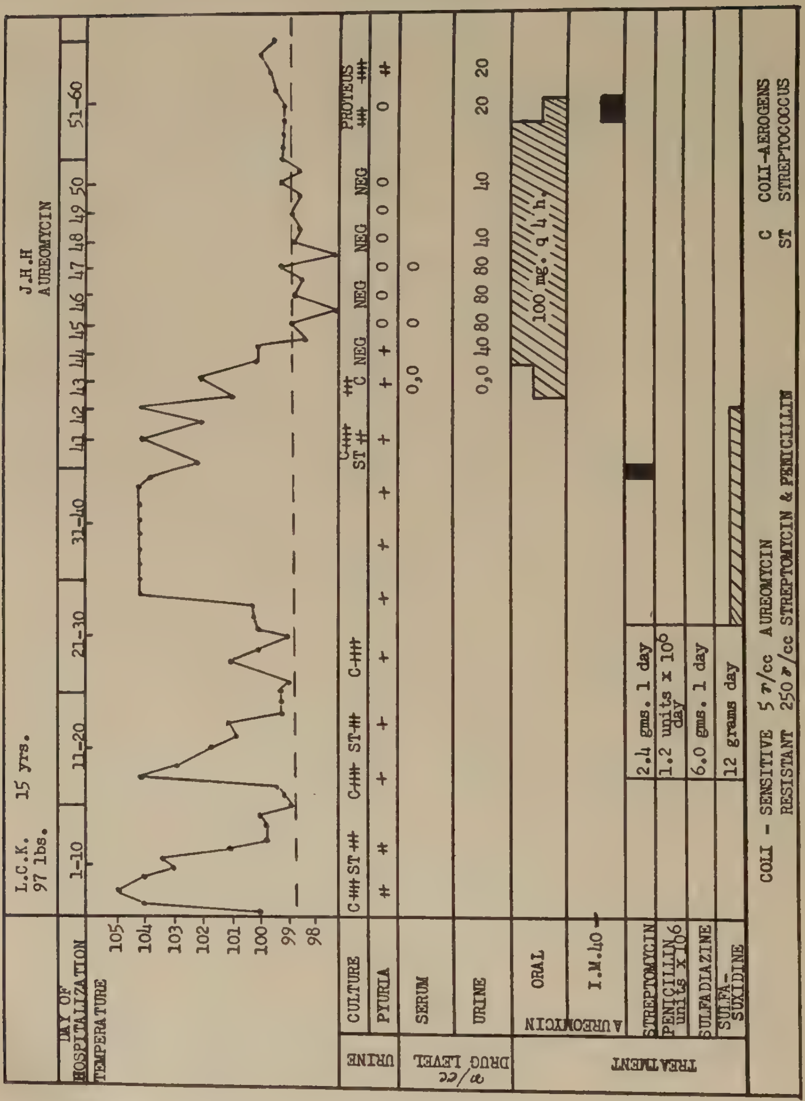


FIGURE 1. Pyelonephritis.

begun on the 43rd hospital day. The temperature promptly fell to normal and cultures of the urine became sterile for the first time. The patient improved in general well-being.

Nine days after aureomycin therapy was begun, *proteus* was noted on a urine culture despite a urinary concentration of 80 micrograms of drug per milliliter. Growth was observed on only one blood agar plate. Two days later, heavy growth of *proteus*, while the patient was on aureomycin therapy, was reported in the culture of urine. Her sedimentation rate

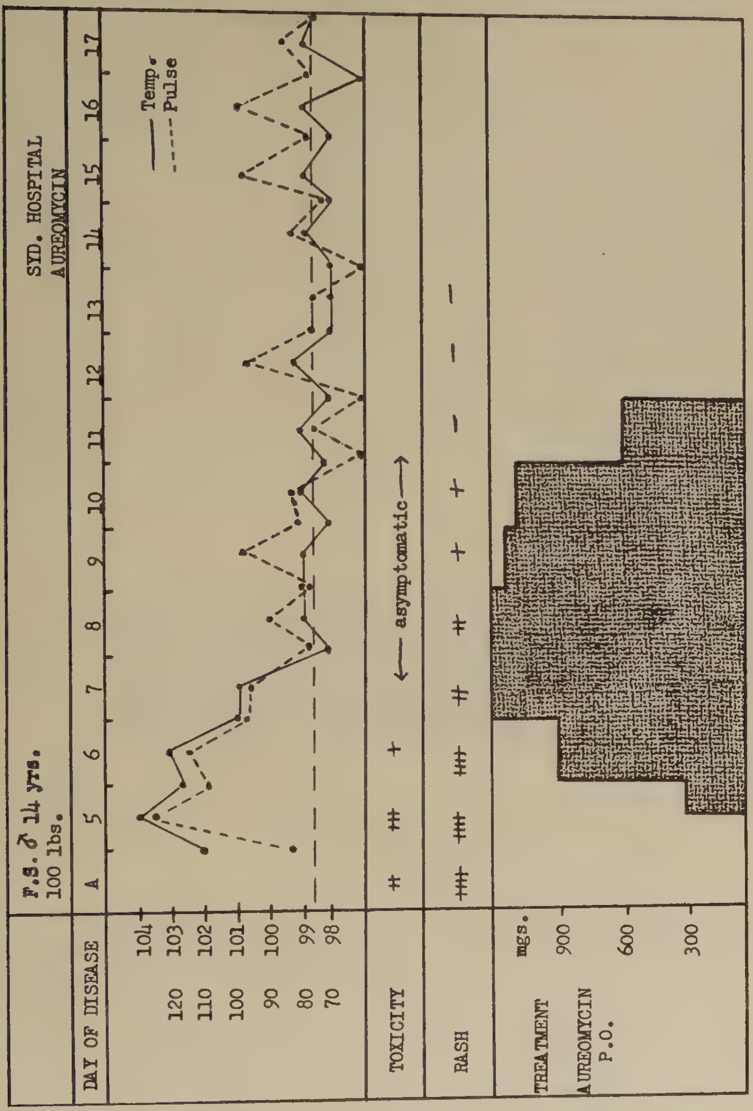


Figure 2. Spotted fever.

became rapid, the white blood count rose to 19,000 cells and low grade fever to 101° appeared. Costo-vertebral angle tenderness, urinary frequency and urgency returned. The urine became alkaline and pyuria reappeared.

Aureomycin was discontinued. The *proteus* infection of the urine persists to date. It is felt that this patient achieved sterilization of her urinary tract infection and symptomatic relief with aureomycin, but that secondary infection with *proteus*, not susceptible to aureomycin, has supervened.

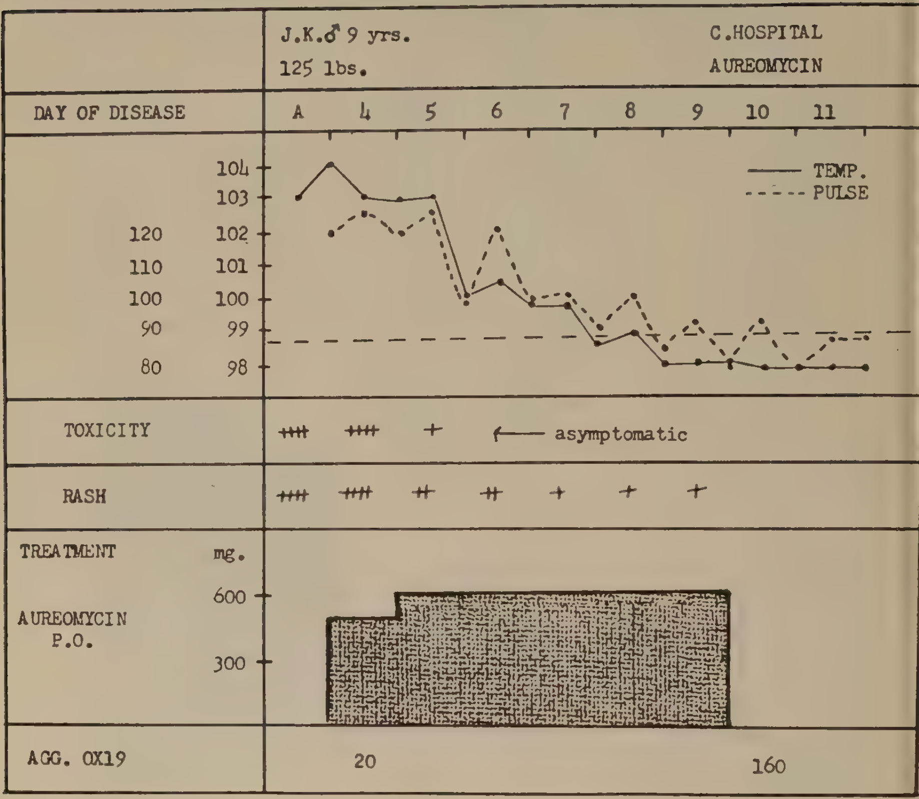


FIGURE 3. Spotted fever.

This patient experienced nausea and occasional emesis while on oral aureomycin therapy. These symptoms were markedly alleviated when amphojel was given with each dose of drug. Urine concentrations of 40–80 micrograms of aureomycin per milliliter were present on the above dosage schedule.

Another patient on the same ward with *coli-aerogenes* pyelonephritis was treated with oral aureomycin. Urinary cultures were sterile and microscopic examination revealed no abnormality within 48 hours after therapy was begun. The drug was discontinued after seven days. A *proteus* infection supervened in this patient two days after the aureomycin was discontinued.

Three cases of Rocky Mountain spotted fever, Eastern type, have been treated with oral aureomycin. Their course is outlined in FIGURES 2, 3 and 4.

A fourteen-year-old boy was admitted to the Sydenham Hospital, Baltimore, Maryland, under the care of Dr. Horace Hodes, Director,

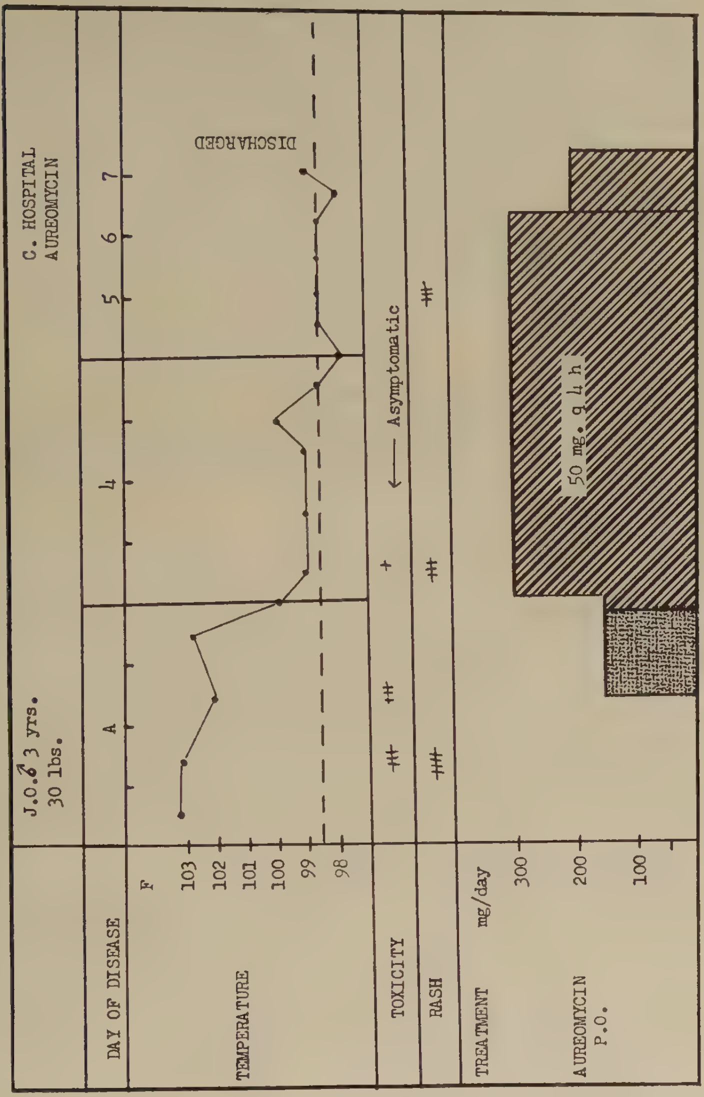


FIGURE 4. Spotted fever.

and Dr. David Karzan. He had been in Virginia during the preceding two months and had been bitten by ticks on numerous occasions. Three days before admission headache, malaise, anorexia, and high fever were noted. Penicillin 300,000 units, sulfadiazine and antipyretic capsules were prescribed. One day before admission, temperature rose to 104° F. and a rash was noted on face and ankles.

Physical examination on admission revealed an acutely ill boy. An erythematous maculopapular eruption with an occasional petechial center was present on ankles, soles, hands, palms, and trunk. A few discrete lesions were present on the face. These lesions blanched somewhat on pressure. Nuchal rigidity was present associated with Kernig and Brudzinski signs.

There was a slight generalized lymphadenopathy and the spleen was palpable two fingerbreadths below the costal margin.

Laboratory data were:

Hemoglobin 15.5 grams per 100 milliliter
Total leucocyte count 2900
Differential leucocyte count
  Polymorphonuclear cells 85%
    Juvenile 11
    Segmented 74
  Lymphocytes 15%
Electrocardiogram—essentially normal
Spinal fluid examination—no abnormality: clear; cells 0; globulin negative.
Urine was clear, amber; specific gravity 1.028 acid and negative tests for albumin, sugar and acetone. The microscopic examination of the sediment was normal.
Blood cultures were negative.
Agglutinations *versus B. proteus* were positive on the fifth day of disease in a dilution of 1/40 or OX_2 and 1/80 for OX_{19}. These rose thirteen days later to OX_2—1/320 and OX_{19}—1/1280.

The temperature rose to 104° F., the day after admission, and oral aureomycin was begun that afternoon. After two initial doses of 100 milligrams given one hour apart, therapy with 100 milligrams every four hours was initiated. The next day, however, the dosage schedule was raised to 100 milligrams every two hours. The temperature fell progressively after drug therapy was begun and was normal within thirty-six hours. Convalescence was uneventful. The drug was stopped on the seventh day of treatment and the patient was discharged one week later. The splenomegaly had disappeared and he felt well.

Blood levels were not measurable in this patient, although the urinary concentration of aureomycin varied between 80 and 320 micrograms per milliliter. No nausea or other untoward symptoms were noted which might be attributed to drug therapy.

Two patients with Rocky Mountain spotted fever, Eastern type, were hospitalized at the Children's Hospital in Washington, D.C. We are indebted to Dr. Sidney Ross, Dr. C. Rice, and Dr. F. Burke of the Antibiotic Study Committee, and the staff at that hospital for their collaboration.

A nine-year-old boy, who had been intimately exposed to ticks in Virginia, became ill two days before admission with fever of 103° F. and abdominal pain. One day later a rash was noted. The child became drowsy and its temperature was maintained at 103° F. During the preceding ten days, a tick had been removed from his body and one from his clothing.

Physical examination revealed a moderately ill, obese boy with a maculopapular rash on abdomen and extremities including the palms and soles. The face was not involved. No lesions were present on the mucous membranes of the mouth or pharynx. The spleen was not palpable and there was no lymphadenopathy. The white blood cell count was 6900 with 63 per cent polymorphonuclear leucocytes. Urine examination was normal, except for a slight albuminuria. Agglutinations were negative, except for the Weil-Felix. Agglutination of *proteus* OX_{19} was positive in a dilution of 1/20 on admission. The latter rose to 1/160 seven days later. The rickettsial complement fixation test performed at the National Institute of Health was reported negative on June 26 and positive in a dilution of 1/64 on July 2, 1948.

Treatment with three doses of 50 milligrams of aureomycin at one hour intervals and 50 milligrams every four hours for three doses was instituted on the third day of his disease. This was then changed to 100 milligrams orally every four hours. The temperature fell by lysis and became normal on the third day of treatment. His general condition improved rapidly and twelve hours after treatment was begun he appeared alert. The drug was discontinued on the sixth day and he was discharged two days later asymptomatic.

A three-year-old infant weighing 30 pounds became ill two days before admission. Drowsiness, malaise, and fever were noted. One day before admission a rash was observed on arms, legs, elbows, and knees. This rash rapidly extended centripetally to involve other parts of the body. A tick had been removed from the right lower extremity, just above the knee, one day before his illness. Many ticks were present in the region and on the farm in Haymarket, Virginia, where he lived.

Physical examination revealed an acutely ill, lethargic infant with a maculopapular rash on all extremities including palms and soles, abdomen, chest, and back. The sclerae were injected and conjunctival bloodvessels dilated. No spleen was palpable. Oral lesions were not present.

Oral aureomycin was begun on the afternoon of admission. Fifty milligrams were given every two hours for the first two doses and thence every four hours day and night for a total period of five days. The temperature fell to normal within twelve hours after therapy was begun and he was discharged as well on the fifth hospital day.

Although none of these cases was desperately ill with signs of coma, vascular collapse, hemorrhage, or edema, it was generally believed that the response to aureomycin was indeed favorable and prompt.

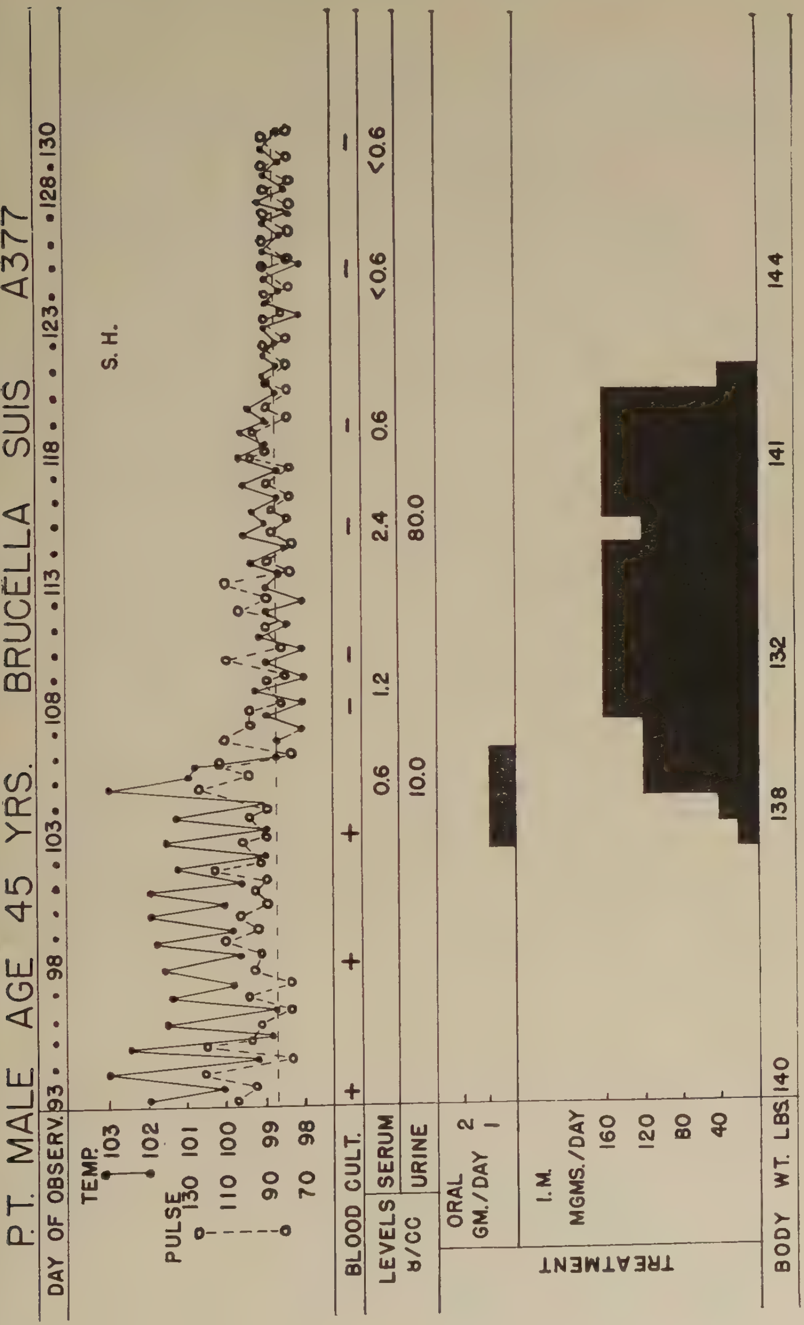
A forty-five-year-old employee of a local meat packing establishment was treated with aureomycin for a persistent infection with *Brucella suis.* In January of 1948, he noted chills and fever which were alleviated somewhat by sulfadiazine. Afternoon chills and evening sweats continued, however, and he was admitted to the Sinai Hospital in Baltimore on February 14, 1948, to the service of Dr. Milton Sherry. Blood cultures were repeatedly positive for *Brucella suis.* Treatment with six grams of sulfadiazine and three grams of streptomycin daily for a period of twelve days was without effect on the clinical course or bacteremia. Polymyxin D was then given for a period of ten days at a dosage of 7½ milligrams per kilogram of body weight per day intramuscularly divided into three doses. Blood cultures became negative promptly and remained negative for a period of four weeks. His general condition improved materially, although a low grade temperature to 100° F. and rapid blood sedimentation rate persisted. He was discharged with weekly follow-up blood cultures and physical examinations recommended.

He was readmitted to the hospital on May 3, 1948, because chills and fever to 104° F. had been noted during the preceding week and headaches, sweating, and general malaise had recurred. A blood culture drawn on April 26, 1948, was reported positive for *Brucella* four days later, although those on April 1, 12, and 19 were sterile.

Physical examination on admission revealed an acutely and chronically ill male. His face was flushed and he was sweating profusely. His liver was enlarged two fingerbreadths below the costal margin; fingers were clubbed. A soft systolic murmur at the cardiac apex was noted by some observers. Petechial and embolic lesions were present on one toe and several fingers. In the region of the right macula, there was a white area interpreted by the ophthalmological consultant as the result of a previous hemorrhage, probably of embolic nature.

Dr. Ronald M. Wood of the laboratories of the Wilmer Ophthalmological Institute, the Johns Hopkins Hospital, has performed all the *Brucella* cultures in this case. He has also collaborated with this department on *in vitro* and experimental animal studies with *Brucella*. *In vitro* tests have shown that growth of five strains of *Brucella*, *abortus* and *suis* types, was completely inhibited by 0.75 micrograms per milliliter of aureomycin. These results were obtained when the final reading was made after seventy-two hours of incubation.

Trial of aureomycin in this case was believed warranted because of the septic course, the persistently positive blood cultures, and failure of sulfadiazine, streptomycin, and polymyxin on previous occasions. After three more positive blood cultures for *Brucella suis* had been obtained, aureomycin therapy was begun. An oral dose of 0.5 grams twice a day was given for four days. This was supplemented by 20 milligrams intramuscularly on the first day, 40 milligrams on the second day, and 120 milligrams divided into three equal doses for the next two days. The oral drug was then discontinued because of anorexia and nausea. It should be noted that the oral drug (lot number 204–214) was relatively crude material. The intramuscular route of administration was maintained for a total of period of twenty days in a dosage of 40.0 milligrams four times each day. The course of this patient while on aureomycin is outlined in FIGURE 5. His temperature became normal four days after therapy was begun. Blood cultures became sterile and have remained negative to date. The last culture drawn one month after discharge from the hospital is sterile. The patient has remained afebrile and asymptomatic. His weight, which had been 140 pounds when first readmitted in May and which had fallen to 132 pounds, is 159 pounds as of July 1. The enlarged liver has regressed



P.T. MALE AGE 45 YRS. BRUCELLA SUIS A377
S. H.
DAY OF OBSERV. 93 98 103 108 113 118 123 128 130
TEMP.
103
102
101
100
99
98
PULSE
130
110
90
70
BLOOD CULT.
+
+
+
-
-
-
-
-
-
LEVELS
SERUM
URINE
γ/CC
0.6
1.2
2.4
0.6
<0.6
<0.6
10.0
80.0
TREATMENT
ORAL
GM./DAY
2
1
I. M.
MGMS./DAY
160
120
80
40
BODY WT. LBS
140
138
132
141
144

Figure 5.

and there has been no evidence of petechial or embolic episodes. The patient has returned to work.

Blood levels while on aureomycin were determined. They varied from 0.6 micrograms per milliliter of serum to 2.4 micrograms per milliliter. Urine concentration of 10.0 to 80.0 micrograms per milliliter were measured.

Nausea and anorexia while on oral drug and local tenderness and induration following the intramuscular injections were the only untoward reactions noted. The patient will be followed for evidence of relapse.

Summary

Aureomycin, a new antibiotic, has been studied in humans. It is irritating upon parenteral administration. Oral administration is well tolerated. The drug is absorbed although blood levels are difficult to determine. It is excreted rapidly in the urine and high concentrations may be attained.

Treatment of pyelonephritis has successfully eliminated the infecting organisms of the *coli-aerogenes* group. *B. proteus* infection of the urinary tract was initiated in the presence of high concentrations of aureomycin and the drug was ineffective in the treatment of this type of urinary tract infection.

Three cases of Rocky Mountain spotted fever, Eastern type, were treated on the third and fifth day of the disease with aureomycin administered orally. All cases became afebrile within 12–72 hours and convalescence was uneventful. The drug was well tolerated in daily dosages up to 30 milligrams per kilogram of body weight. A case of *Brucella suis* infection which had not responded to sulfonamide and streptomycin therapy and which had relapsed after polymyxin therapy has become asymptomatic after treatment with oral and intramuscular aureomycin.

No toxic or allergic reactions have been observed in patients or normal individuals except for transient nausea. These may only become apparent when a larger series is treated. The preliminary results with aureomycin to date have been encouraging and more extensive clinical trial is indicated.

Addendum

Since this paper was submitted, a total of 16 cases of Rocky Mountain spotted fever have been treated with oral aureomycin. Treatment was begun on the 2nd to 8th day of disease (average 4.5) and normal temperature and clinical cure was noted 36 to 72 hours after the institution of treatment (average 2.3 days). No complications or deaths occurred. Thirteen cases in this group have been confirmed serologically with Weil-Felix and/or specific complement fixation tests.

Two additional cases of acute brucellosis have been treated, with bacteriological and clinical remission within 72 hours.

Three early cases of typhoid fever treated with aureomycin in daily dosage of 60–100 mg./kg. per day by mouth and 3.0 to 5.0 mg./kg. per day parenterally have resulted in negative blood and stool cultures within 48 to 72 hours. Clinical response has varied, however, with defervescence occurring in 24 hours, 8 days, and 11 days respectively.

Five cases of newborn infants with multiple skin and breast abscesses due to hemolytic *Staphylococcus aureus* cleared rapidly on oral aureomycin. One of these cases also had staphylococcal infection of the lung with abscess formation and positive blood culture.

Four cases of primary atypical pneumonia have responded to oral aureomycin with prompt defervescence within 36 to 48 hours. Convalescence was uneventful.

A thirteen-year-old girl with tuberculous draining sinuses of the neck for 5 years was treated with a total of 92 grams of aureomycin orally for a period of seven weeks. Drainage ceased within 10 days and nodes decreased in size. No recurrence has been noted, although drug therapy has been discontinued for a period of three weeks.

Eight additional cases of chronic urinary tract infection which had not responded to penicillin, streptomycin or sulfonamide therapy have cleared promptly with aureomycin. The organisms identified in these cases were *B. coli-aerogenes*, *B. paracolon* and *Streptococcus fecalis*.

One case of epidemic typhus (Brill's disease) treated on the 6th day of his disease became afebrile and asymptomatic within 48 hours.

AUREOMYCIN IN OCULAR INFECTIONS

A Study of Its Spectrum

By Alson E. Braley and Murray Sanders

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AUREOMYCIN is an antibiotic obtained from a mold belonging to the *Streptomyces* group. It was isolated by workers at the Lederle Laboratories of the American Cyanamid Company.[1] This antibiotic was found by these workers to be effective against both gram positive and gram negative organisms, and their work suggested a wide range of activity. It has been made available to us in two forms, Aureomycin hydrochloride and Aureomycin borate.

Aureomycin HCl is a dry, crude, crystalline substance, yellow in color. When in aqueous solution, it is an acid with a pH of 3, and is stable either in the dry form or in solution without refrigeration. In solution, it can be injected intramuscularly. The dry material is quite soluble in water or normal saline; 20 mg. will dissolve in 1 cc. to 1.5 cc. of normal saline. This material is moderately irritating when injected intramuscularly, but with the addition of a small amount of procaine hydrochloride there is little or no discomfort.

A borated salt of Aureomycin was also manufactured and dried into a fine, yellow, stable powder. In aqueous or physiological saline solution the pH is 7.5 to 7.8. It is very soluble in nearly all concentrations; however, in solution at room temperature the material loses its antibiotic activity within approximately 24 hours. Various concentrations of the borate were used, but is was found that a ½ per cent solution was the one best tolerated. The activity of the ½ per cent solution is retained for several days when it is stored at +4° C. Freshly prepared Aureomycin borate solution is a light golden-yellow color. After it has remained at room temperature for 24 hours it turns a golden-brown.

Wong and Cox[2] have shown in experimental infections with a wide range of microorganisms, rickettsiae, and viruses that Aureomycin was unusually potent and active. Since this was true experimentally, it was felt that this new antibiotic should be tried in many types of ocular infections. The antibiotic was used in this study much the same as penicillin might be used clinically. The borated salt was used locally in eyes for infections of the conjunctiva and cornea. When infection was present in the deeper structures of the eye, such as uveitis and in ocular infections associated with general disease, the local application was augmented by intramuscular injections of Aureomycin HCl.

In a previous report, 100 patients with conjunctival and corneal infections have been reported.[3] It was found from this study that local use of Aureomycin borate effectively inhibited staphylococci, pneumococci, and *H. influenzae.* It was also active against the virus of inclusion conjunctivitis, and appeared to have some value in herpes simplex corneae and to a lesser extent in epidemic keratoconjunctivitis. Since that time, additional information has been obtained regarding these same organisms as well as other ocular infections. The present study was made in an attempt to find the number of diseases and microorganisms in which Aureomycin might be effective. The use of Aureomycin in 200 unselected cases showed that the antibiotic was effective in the treatment of 158 and of no value in 42. This can be further separated into 189 cases in which the infection was primarily of the conjunctiva and cornea, and 11 cases in which the infection was primarily of the uvea (see TABLE 1).

TABLE 1

RESULTS OBTAINED IN AUREOMYCIN TREATMENT OF 200 OCULAR INFECTIONS

Infection	*No. of cases*	*Clinical cure*	*No improvement*
Conjunctivitis			
Staphylococcus aureus			
mild	22	21	1
severe	74	73	1
D. pneumoniae	5	5	
H. influenzae	4	4	
Moraxella lacunata (diplobacillus of Morax-Axenfeld)	5	4	1
E. coli	1	1	
Follicular (etiology unknown)	14	14	
Inclusion conjunctivitis	5	5	
Trachoma	1	1	
Vernal	6	2	4
Epidemic keratoconjunctivitis	27	8	19
Molluscum contagiosum	1		1
Parinaud's conjunctivitis (leptotrichosis)	1		1
Keratitis			
Dendritic (herpes simplex)	6	5	1
Unclassified (etiology unknown, probably infectious)	7	3	4
Acne rosacea	3	1	2
Superficial punctate (virus?)	2	1	1
Neurotropic	2		2
Marginal, severe	1	1	
Pinguecula	1		1
Episcleritis	1		1
Uveitis			
Idiopathic	5	4	1
Lymphogranuloma	1	1	
Scrafuloderm with uveitis and keratitis	2	2	
Sympathetic ophthalmia	2		2
Endophthalmitis, metastatic	1	1	

Staphylococcal Infections. Ninety-six patients with various types of staphylococcal infections were treated. The majority had blepharitis combined with a conjunctivitis and keratitis. There were, however, a fair number of cases with marginal infiltrates and marginal ulcers associated with the chronic conjunctivitis. A few patients had horeola and chalazia complicating the blepharitis. For statistical purposes, we divided staphylococcal infections into mild and severe cases. There were 22 patients in the group considered mild, those with a blepharitis squamosa and a chronic conjunctivitis associated with a slight discharge. Severe staphylococcal infections were those with a blepharitis with an acute or subacute conjunctivitis combined with corneal changes such as superficial punctate keratitis, marginal infiltrates, or marginal ulcers. The evaluation of the results of Aureomycin in staphylococcal infections was difficult because of the tendency for recurrence of all types of staphylococcal infections and the inclination of the patient to discontinue treatment as soon as the acute symptoms subsided. In order to evaluate the effectiveness of the antibiotic, an arbitrary 48 hours was chosen as the time limit for disappearance of symptoms. If there was no improvement in the objective appearance or symptoms, the action of the antibiotic was considered unfavorable. There is little doubt, as reported in the previous publication, that Aureomycin is very active against the staphylococcus, whether the disease produced by it is mild or severe. From experience with these patients, it seems to be as potent as local penicillin (1,000 units per cc). Staphylococcal conjunctivitis had a tendency to recur when therapy was discontinued. Recurrences were about the same with Aureomycin as with other local antibiotics. Aureomycin was potent in patients with staphylococcal conjunctivitis who had developed a sensitivity to the local use of penicillin.

Staphylococcal infections require local therapy for a long period of time and since a transition occurs in Aureomycin borate solution, an attempt was made to prepare an ointment with both the hydrochloride and the borate. The hydrochloride ointment was irritating to nearly all of the patients, although it seemed to be active against the infection. The ointment prepared with the borate was non-irritating, but it probably lost its activity after a short period. Further investigation will be necessary to determine the best means of retaining this antibiotic effect for the required prolonged use in staphylococcal infections.

There were only two unfavorable responses to Aureomycin in the entire group. The organisms could have been assayed to determine the amount of the antibiotic necessary to inactivate them, but it is possible that the patients did not use the antibiotic as directed.

Pneumococcal Conjunctivitis. The second largest group of patients with bacterial conjunctivitis was infected by the pneumococcus. Only 5 patients with pneumococcal conjunctivitis have been treated; there were excellent results and no recurrences.

Influenzal Conjunctivitis. The response of 4 patients from whom influenza bacilli were cultured from the conjunctiva was rapid and efficient. In all of the patients, the purulent discharge was entirely gone within

24 hours. It was difficult to evaluate the antibody response to influenzal conjunctivitis, since the duration of this infection without treatment varied considerably. From the appearance of the conjunctiva, however, we felt that Aureomycin was potent against *H. influenzae*. Further investigation will be necessary, particularly in influenzal meningitis, to determine the degree of potency.

Diplobacillary Infections. The diplobacillus of Morax-Axenfeld has been an organism which has not responded well to any form of therapy. In 4 cases the symptoms were relieved and no bacteria found on scrapings of the conjunctiva after local use of Aureomycin. In one patient, a recurrence developed in one eye shortly after the antibiotic was discontinued. From all clinical appearances, however, Aureomycin is much more effective against the diplobacillus than any other therapy.

E. COLI. Aureomycin borate was used in only one case of infection of the conjunctiva by E. coli. A pure culture of *E. coli* was obtained from the conjunctiva after several months of conjunctivitis. Following the use of this antibiotic for 24 hours, conjunctival symptoms were entirely gone and the conjunctiva was free of organisms.

Viral Infections. Two viruses involving the conjunctiva and cornea have been treated with Aureomycin, 6 cases of dendritic keratitis, and 27 cases of epidemic keratoconjunctivitis. Clinically, Aureomycin appears to be more useful in the herpes corneae virus than in epidemic keratoconjunctivitis.

Dendritic Keratitis. All 6 of the patients with dendritic keratitis had an associated beginning involvement of the corneal stroma with an area of infiltration under the dendritic figure. In 5 of these patients, the results after use of Aureomycin were rapid and beneficial. The ulcer was healed in 24 hours and there was no increase in the size of the infiltrate beneath the ulcer, and in most of the patients the infiltrate disappeared entirely. In one patient who had a recurrent dendritic keratitis with a large disciform keratitis there was no appreciable change in the involvement of the corneal stroma after Aureomycin. From our experience thus far with the antibiotic, it seems to be a valuable therapeutic agent for dendritic keratitis.

Epidemic Keratoconjunctivitis. The use of Aureomycin in the treatment of epidemic keratoconjunctivitis does not appear effective from the data presented. Twenty-seven patients with the infection have been followed after the use of the antibiotic. There were many more patients with epidemic keratoconjunctivitis who had used Aureomycin, but their follow-up was not entirely satisfactory and they cannot be included in the series. The course of the disease was not affected in 19 of the 27 patients, but 8 showed a definitely favorable reaction. These patients taught us a good deal as to how this new antibiotic should be used. Certainly in epidemic keratoconjunctivitis, Aureomycin must be used before corneal opacities begin, since in several of the 19 patients it was started after corneal opacities were first noted. It appears that if Aureomycin can be started before the third to fifth day of the disease and used continually for at least a week or ten days there is some beneficial effect. If the material is instilled in the conjunctival sac every one-half to one hour and continued, even though the symptoms and edema of the conjunctiva increase, there will certainly be some favorable results. Most of the 8 favorable cases developed one or two typical corneal opacities at about the same time as they would had the disease been allowed to run its normal course, but the conjunctival findings and the corneal changes were considerably decreased as compared to control cases. Even though Aureomycin was used, a few developed conjunctival scars and minimal symblepharon. Patients who used the antibiotic a few times and discontinued its use after 24 or 48 hours because of its slight irritability received no benefit from the antibiotic. The method of choice, therefore, in treatment of epidemic keratoconjunctivitis should be the instillation of Aureomycin borate every hour for at least ten days with a fresh supply of the antibiotic given at 48-hour intervals. Blood has been obtained from the patients for neutralization of the virus to determine the presence of antibodies.

Follicular Conjunctivitis, Unknown Etiology. The evaluation of the use of Aureomycin in follicular conjunctivitis is rather difficult, since this group contains a number of patients with what clinically appeared to be a Beal's type of conjunctivitis. This type of follicular conjunctivitis is notoriously inconsistent in the duration of symptoms. With Aureomycin, however, none of the cases of follicular conjunctivitis lasted more than 48 hours.

Inclusion Conjunctivitis and Trachoma. Local Aureomycin was used in 5 cases of inclusion conjunctivitis. Most of these were in the new-born infant after the disease had been present for from five days to two weeks. The response to Aureomycin was prompt and the purulent discharge was entirely gone after 24 hours of its use. The conjunctiva returned to normal within three days to one week. Some of the cases have been followed with daily scrapings from the conjunctiva; no inclusion bodies could be found after 24 hours on Aureomycin.

One case of trachoma III which had been recurrent for twenty years was treated. This patient had developed anuria on sulfanilamide. Following treatment with local Aureomycin there was rapid disappearance of corneal infiltrates and conjunctival symptoms. No inclusion bodies could be demonstrated from the conjunctival scrapings. From the effect of Aureomycin on inclusion conjunctivitis and lymphogranuloma, we feel that it should also be effective in trachoma.

Molluscum Contagiosum. One patient with molluscum contagiosum has been treated with Aureomycin locally. There was no effect on the tumor or on the appearance of the conjunctiva.

Keratitis. Fifteen cases of various types of keratitis have been treated. Some of these have been remarkable, particularly one patient with a severe marginal keratitis of unknown etiology. This patient had marked thinning of the periphery of the cornea, and the central cornea was necrotic. His vision was reduced to hand movements in one eye and 20/400 in the other eye. After local and parenteral Aureomycin the process stopped and the central portion of the cornea became clear while the periphery vascularized. The vision was restored to 20/25 in one eye and 20/20 in the other.

Three cases of acne rosacea keratitis have been treated, one with some beneficial effect and two without any effect. It is doubtful that any of the various types of keratitis profunda or severe types of keratitis are particularly benefitted by Aureomycin although 6 of the 15 cases improved under Aureomycin therapy.

Two patients with neurotropic type of keratitis were unaffected by Aureomycin.

Parinaud's Conjunctivitis. Only one patient with Parinaud's conjunctivitis was treated with Aureomycin with no effect on the progress of the disease.

Vernal Conjunctivitis. Six cases of vernal conjunctivitis have been treated with varying amounts of local Aureomycin. Four were unchanged, but two showed a disappearance of the filmy membrane and some relief of symptoms, although there was no change in the papillary hypertrophy in the conjunctiva. It is probable that the improvement was due to the antibiotic action on the secondary infection.

Uveitis. All of the patients with uveitis have been treated with local borate salt and intramuscular Aureomycin HCl. Of the 11 patients with various types of uveitis, 8 have shown beneficial results, while 3 have remained unchanged. The most striking in this group are 2 patients with scrafuloderm combined with a keratitis and uveitis. Not only was the eye restored to normal vision, but there was also marked improvement in the draining sinuses and skin reaction associated with the underlying lymph node disease. In one of these patients, tubercle bacilli were demonstrated in biopsies of the lymph nodes, and in the other, the biopsy and guinea pig inoculation was unsatisfactory. There are under observation at the present time two other patients with tuberculous lymphadenopathy and keratitis. The response of the cornea has been prompt, but there has been no change in the lymphadenopathy so that these cases have not been included in this study. The response to Aureomycin in these cases is certainly as satisfactory as with streptomycin.

One patient with a recurrent unilateral uveitis in whom a positive Frei test was found, responded dramatically to treatment with Aureomycin. She developed a recurrence two weeks after receiving 100 mg. intramuscularly and was given another course of 100 mg. with complete recovery.

Five cases of uveitis of unknown etiology have received Aureomycin; 4 have shown definite improvement while one has remained unchanged. Several more patients are under observation at the present time, but it is too early to draw any conclusions as to the efficiency of the antibiotic.

Two cases of sympathetic ophthalmia were given Aureomycin without any beneficial result, although one patient insisted on receiving it for a long period of time because he stated that his eyes felt better. There was no change in the clinical appearance of the eye.

One patient with a metastatic endophthalmitis of unknown etiology has been treated. She had developed a large abscess in the vitreous hump with pus in the anterior chamber following considerable abdominal surgery. She was given 300 mg. intramuscular Aureomycin and local drops after which all of the pus disappeared. A red reflex could be obtained from the fundus, but because of the vitreous opacities it was impossible to see the fundus details at the time of her discharge from the hospital. The eye has remained quiet since that time.

Comments

We have previously reported on the antibiotic properties of local Aureomycin in staphylococcal, pneumococcal, and influenzal conjunctivitis. To this group we are adding the diplobacillus of Morax-Axenfeld and *E. coli*. Several diseases of unknown etiology were treated in an attempt to determine the spectrum of the antibiotic. The validity of Aureomycin in some of the virus infections was not anticipated. From the experiments of Wong and Cox[2] we learned that it should be of value in the psittacosis-lymphogranuloma group and in rickettsial diseases. In our experience, Aureomycin was excellent in the treatment of inclusion conjunctivitis. Because of the similarity between the virus of trachoma and the virus of inclusion, it should be a useful therapy for trachoma (see TABLE 2).

Aureomycin seems to have some antibiotic properties to the virus of herpes simplex. The results of its use on herpes infections of the cornea were striking. Experimental herpes simplex infections will be reported later, although at the present time they indicate that Aureomycin has some anti-viral properties.

Its use in epidemic keratoconjunctivitis is not entirely satisfactory, but there is a strong indication from several patients that if the antibiotic is used properly, considerable benefit may be expected. After Aureomycin borate had been used for a period of 48 hours, it became somewhat irritating to the conjunctiva, in cases of epidemic keratoconjunctivitis particularly, and many patients thought it produced a more severe conjunctivitis. We found that the applications of the antibiotic must be continued in spite of increased symptoms in order for it to be beneficial. There is no change in epidemic keratoconjunctivitis much short of a week. If the material was used conscientiously by the patient,

TABLE 2

OCULAR INFECTIONS WHICH SHOULD RESPOND WELL TO AUREOMYCIN THERAPY

Virus infections:
- inclusion conjunctivitis*
- trachoma*
- lymphogranuloma venereum**
- herpes simplex corneae*
- follicular conjunctivitis*

Hemolytic streptococcus infections:
- conjunctivitis** (membranous)
- corneal ulcers**
- endophthalmitis**
- orbital cellulitis**
- impetigo*

Staphylococcus infections:
- dacryocystitis
- conjunctivitis*
- ulcers*
- endophthalmitis**
- blepharitis*
- orbital cellulitis
- impetigo*

Pneumococcus infections:
- dacryocystitis
- conjunctivitis*
- ulcers**
- endophthalmitis
- orbital cellulitis

Gonococcus infections:
- conjunctivitis
- iridocyclitis

B. pyocyaneus infections:
- ulcers

B. proteus infections:
- conjunctivitis

Coliform group infections:
- conjunctivitis*

H. influenzae infections:
- conjunctivitis*
- ulcers**
- orbital cellulitis

Diplobacillus (*Morax-Axenfeld*)
- conjunctivitis*
- ulcers**

Friedlander bacillus infections:
- ulcers**
- conjunctivitis*
- meibomitis**
- dacryocystitis

Meningococcus infections:
- endophthalmitis
- conjunctivitis

* Local therapy preferred.
** Combined intramuscular and local therapy preferred.

TABLE 3

OCULAR INFECTIONS IN WHICH AUREOMYCIN MAY BE OF VALUE AND DESERVES TRIAL

Virus infections:
- epidemic keratoconjunctivitis*
- herpes zoster**
- herpes simplex corneae*

Tuberculosis:
- conjunctivitis* (ulcers)
- uveitis**
- keratitis
- scrafuloderm**
- kerato-uveitis

Non-hemolytic streptococcus infections:
- orbital cellulitis
- endophthalmitis
- corneal ulcers

Brucella melitensis-abortus-suis:
- keratitis
- uveitis
- choroiditis

Moraxella duplex (*diplobacillus of Petit*):
- central ulcers

H. Ducreyii
- soft chancre of lid or conjunctiva

Syphilis:
- chancre of lid
- choroiditis
- optic atrophy

Keratitis (marginal) unknown etiology

Uveitis (idiopathic)

* Local therapy preferred.
** Combined intramuscular and local therapy preferred.

the period of morbidity was shortened from approximately three weeks to a period of ten days. The corneal opacities which developed were usually minimal as compared to control cases (see TABLE 3).

Aureomycin had no effect on the virus of molluscum contagiosum.

Aureomycin was surprisingly effective in 14 cases of follicular conjunctivitis. In none of these was it possible to determine the etiology.

Six cases of vernal conjunctivitis were treated and no particular result was anticipated. It is well known that any of the antibiotics will have some tendency to give a certain degree of subjective improvement, and it is doubtful if Aureomycin will have any effect on vernal conjunctivitis.

Although one case of Parinaud's conjunctivitis due to the leptothrix was treated, no beneficial effect was anticipated because of the similarity between the leptothrix and the actinomyces form which Aureomycin is made.

In the treatment of uveitis and keratitis, it was not possible to determine in advance which patients would be improved. There is little doubt in our minds that there was marked improvement in the patients with scrafulous keratitis and scrafuloderm. There is also little doubt that the antibiotic produced improvement in several cases of uveitis of unknown etiology.

TABLE 4 lists some infections in which Aureomycin is not expected to be of value.

TABLE 4

OCULAR INFECTIONS IN WHICH AUREOMYCIN PROBABLY IS OF NO VALUE

Erythema multiforme:
    conjunctivitis
    keratitis
Ocular pemphigus
Parinaud's conjunctivitis:
    leptotrichos

Sympathetic ophthalmia
Vernal conjunctivitis
Molluscum contagiosum
Mooren's ulcer
Streptothrix concretions

Summary

Aureomycin borate has been used locally, and Aureomycin HCl has been used intramuscularly in 200 patients with a wide range of ocular infections. The local use of ½ per cent solution produced no damage to the conjunctiva or cornea. This antibiotic was found to be effective against some of the gram positive cocci and several gram negative bacilli. It was also found to be an effective therapeutic agent in inclusion conjunctivitis and in herpes simplex of the cornea. Its therapeutic effect in epidemic keratoconjunctivitis will require further investigation before results can be evaluated. It is, however, more effective in epidemic keratoconjunctivitis than any of the other antibiotics or drugs tried. The intramuscular administration of Aureomycin HCl did not give rise to any toxic reactions and in only one individual was any general effect noted. This patient developed a secondary anemia which was easily controlled by the administration of iron. The HCl is somewhat irritating on intra-

muscular injection, but this irritation can be controlled by the addition of a small amount of procaine hydrochloride. There is some indication that Aureomycin may be a valuable antibiotic in the treatment of uveitis.

Aureomycin has a wide spectrum of activity in ocular infections.

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ACTION OF AUREOMYCIN AGAINST EXPERIMENTAL RICKETTSIAL AND VIRAL INFECTIONS

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TIME does not permit for a review of the literature on the treatment of viral and rickettsial infections with chemical and antibiotic substances. The results of such investigations have been reported recently by several groups of workers.

In this preliminary report are presented some of the data obtained with the newly described antibiotic, Aureomycin, in experimental infections of embryonated eggs, mice, and guinea pigs with the agents of lymphogranuloma venereum, psittacosis, murine typhus, epidemic typhus, Rocky Mountain spotted fever, Q fever, rickettsialpox, and scrub typhus.

Materials and Methods

In most instances the antibiotic was resuspended in one-twentieth molar phosphate buffer pH 7.0. Buffered suspensions of this type, containing 5 mg. of Aureomycin per ml., have a final pH of 6.8. Any solution left over was usually discarded, although subsequent studies indicate that if the unused material is frozen immediately, the potency appears unimpaired for at least 2 weeks.

Chemotherapy Experiments in Eggs. The following method was used to determine if the chemotherapeutic agent under investigation showed antiviral or antirickettsial activity. Seven-day-old embryonated hens' eggs were inoculated into the yolk sac with 0.2 ml., containing the desired dosage of Aureomycin. The control group received the same amount of buffered saline. Thirty minutes later all groups of eggs were reinjected into the yolk sac with 0.1 ml. of the various ten-fold dilutions of infectious inoculum. All dilutions of the infectious inoculum were made with cold 0.1 per cent casein solution, pH 7.0, and all inoculum dilutions were kept refrigerated until used. All eggs were sealed with collodion, stored in an incubator at 96° F. and candled at least once a day for a total of 13 days.

In most instances, deaths of embryos before the fourth day were considered to be non-specific and were not included in the analysis of the results. All embryos dying on the fourth day and thereafter were thoroughly examined for the presence of elementary bodies or rickettsiae, using yolk-sac or amniotic-sac smears stained by the Macchiavello method. All embryos surviving after the 13th day were examined in the same manner.

Chemotherapy Experiments in Mice. Swiss albino mice, weighing from 14 to 16 grams, were infected by the intracerebral or intraperitoneal route with 0.03 ml. or 0.25 ml., respectively, of an appropriate dilution of infectious inoculum. Aureomycin was administered to mice by mouth or injected subcutaneously, usually in a single dose given once a day. Mice treated orally were given the drug by means of a 1 or 2 ml. tuberculin syringe fitted with a round-end 18 gauge needle, 1½ inches long. The mice were grasped firmly by the back of the head and turned over with the head, slightly elevated, held away from the operator. The full length of the needle was slid slowly past the esophagus into the stomach and the required amount of Aureomycin was then given.

Chemotherapy Experiments in Guinea Pigs. Guinea pigs, weighing 450 to 500 grams, were injected intraperitoneally with 1 ml. each of the appropriate dilution of infectious inoculum. All dilutions were made with 0.1 per cent casein solution pH 7.0. Rectal temperatures were taken for 5 days prior to infection and were continued for at least 2 weeks post infection. Aureomycin was given subcutaneously, as a rule once a day, and in most cases consisted of a 3 mg. dose in 1.5 ml. amounts.

Results

Table 1, which represents a typical experiment carried out in embryonated hens' eggs, shows the results obtained with Aureomycin in psittacosis infection. A single injection of 1 mg. of Aureomycin was given

Table 1

PSITTACOSIS
IN EMBRYONATED EGGS

DILUTION OF INOCULUM	SURVIVAL RATIO ON 13TH. DAY		PRESENCE OF M. PSITTACII BY MICROSCOPIC EXAMINATION	
	TREATED*	CONTROLS	TREATED	CONTROLS
10^{-1}	8/8		0/8	
10^{-2}	9/9		0/9	
10^{-3}	9/10	0/10	0/10	10/10
10^{-4}	8/9	0/10	0/9	10/10
10^{-5}	8/10	0/10	0/10	10/10
10^{-6}	7/9	0/9	0/9	10/10
10^{-7}		0/9		10/10
10^{-8}		3/9		5/9

* A SINGLE INJECTION OF 1 MG. OF DRUG GIVEN 30 MINUTES PRIOR TO INOCULATION

into the yolk sac 30 minutes prior to inoculation with serial ten-fold dilutions of psittacosis (6 BC strain) infected yolk sac. The results show that 49 of the 55 treated embryos survived the infection through the 13th day, whereas only 3 of 57 control embryos survived. That the protection afforded was not border-line is shown by the fact that the drug protected all embryos that received the 10^{-1} and 10^{-2} dilutions of infectious inoculum, equivalent approximately to 10 million and 1 million lethal doses, respectively. Secondly, it is seen that it was not possible to demonstrate elementary bodies in any of the drug treated embryo yolk sacs, although 55 of the 59 control embryos were positive.

The results shown here were found to hold true essentially for all the viruses of the psittacosis-lymphogranuloma group (lymphogranuloma venereum, psittacosis, SF strain human pneumonitis, mouse pneumonitis, feline pneumonitis, and meningo-pneumonitis F97 strain), as well as the rickettsiae of murine typhus, epidemic typhus, scrub typhus (Karp strain), Rocky Mountain spotted fever, boutonneuse fever, South African tick-bite fever, rickettsialpox, North Queensland tick typhus, and Q fever (original Montana Nine-Mile strain, California bovine strain, the Dyer strain, Lynn strain, Italian Henzerling strain, and two Swiss strains).

It should be pointed out that although no elementary or rickettsial bodies could be demonstrated microscopically in the yolk sacs of Aureomycin-treated eggs in any of the above named infections, when 4 or more yolks sacs from living treated-embryos that received the more concentrated inocula were pooled to make a 10 per cent suspension and injected into other embryonated eggs, elementary or rickettsial bodies could be demonstrated in most instances in the yolk sacs of the first passage eggs. Hence, it is apparent that chick embryos that received massive infecting doses were not entirely freed of the infectious agent by a single injection of Aureomycin. In all probability a quantitative relationship exists between the dosage of the infecting agent and the dosage of Aureomycin used. Further experiments along these lines are in progress.

More recent experiments with psittacosis and epidemic typhus have shown that treatment of infected embryonated eggs with Aureomycin may be delayed as long as 24 hours post infection with marked beneficial results.

Effect of Aureomycin IN VITRO. In experiments carried out with epidemic typhus, murine typhus, Q fever and psittacosis, it was found that Aureomycin showed little or no direct virucidal or rickettsiacidal activity. This was demonstrated in experiments in which 2 portions of the infectious agent under test were prepared; one contained the infectious agent suspended in 0.1 per cent casein solution pH 7.0, whereas the other contained the infectious agent in 0.1 per cent casein solution in which was dissolved 1 mg. of Aureomycin per ml. The final concentration of infected yolk-sac suspension in the mixtures varied from 1:100 to 1:1000. The mixtures were held at room temperature for 30 minutes and then titrated in embryonated eggs. In all instances the LD_{50} titers of the two portions were practically identical. In experiments of this type, virucidal or rickettsiacidal activity apparently was observed only in those eggs that received the original mixture in which the antibiotic was present in highest concentration.

Table 2 shows the results obtained when mice were injected intraperitoneally with approximately 47,000 LD_{50} of psittacosis infected yolk-sac inoculum and treated with Aureomycin by the subcutaneous route with 1 mg. per day for 1, 3, 5, and 7 days, starting treatment 24 hours after infection. The results show that a minimum of 3 injections of 1 mg. each daily were required to give complete protection to the treated mice. However, a single injection of 1 mg. showed some protection since 4 out of 10 mice survived in this group, whereas all the controls died.

Table 2

PSITTACOSIS

INOCULATION OF YOLK-SAC SUSP.		TREATMENT* 1 MG. DAILY	SURVIVAL RATIO OF MICE 17 DAYS AFTER INOC.	
ROUTE	DILUTION	NO. OF DAYS	TREATED	CONTROLS
I. P.	10^{-3} **	1	4/10	
		3	10/10	
		5	9/10	
		7	8/9	
				0/10

* TREATMENT STARTED 24 HOURS AFTER INOCULATION

** $10^{-3} = 47000\ LD_{50}$

Table 3 shows the results obtained when mice were injected *intracerebrally* with graded doses of lymphogranuloma venereum virus and treated with Aureomycin by the subcutaneous route with 1 mg. per day for 7 days, starting 24 hours post infection. It is seen that 39 of the 40 mice treated were completely protected, whereas all 39 mice receiving the same infectious dilutions died. Similar results have been obtained with mice infected intracerebrally with the 6 BC strain of psittacosis virus. These findings are noteworthy in view of the fact that Smadel and Jackson (1948) reported that Chloromycetin shows no beneficial effect in mice infected intracerebrally with either of the above viruses. These results indicate that Aureomycin is more active and that it must be able to pass the blood-brain barrier in order to protect mice against intracerebral infection with the psittacosis-lymphogranuloma group of viruses.

TABLE 3

LYMPHOGRANULOMA VENEREUM

INOCULATION OF YOLK-SAC SUSP.		TREATMENT* -- DAILY	SURVIVAL RATIO OF MICE	
ROUTE	DILUTION	MG.	TREATED	CONTROLS
I.C	10^{-2}**	1.0	9/9	0/11
	10^{-3}		10/10	0/9
	10^{-4}		11/11	0/9
	10^{-5}		9/10	0/10
	10^{-6}			4/10

*TREATMENT STARTED 24 HOURS AFTER INOCULATION AND CONTINUED FOR 7 DAYS

** 10^{-2} = 13000 LD_{50}

TABLE 4 shows the results obtained when mice were injected intracerebrally with approximately 500 LD_{50} of lymphogranuloma venereum virus and treated with Aureomycin by the subcutaneous route with 1 mg. per day for 3 days, starting treatment 24, 48, 72, and 96 hours post infection. It is seen that of the 10 active mice treated within 24 hours, all survived. Those treated at 48 hours, at which time 2 were sick and 6 were active, all survived. With those treated at 72 hours, at which time all 9 were sick, 7 of the 9 survived. Those treated at 96 hours, at which time all 8 had been sick for 2 days, only 1 of the 8 survived. The results obtained in the last group bring up the question—would more mice have been saved had the daily dosage been increased or had the number of doses per day been increased?

TABLE 4

LYMPHOGRANULOMA VENEREUM

INOCULATION OF YOLK-SAC SUSP.		TREATMENT* STARTED	NO. OF MICE IN GROUP		SURVIVAL RATIO
ROUTE	DILUTION		AT START	TIME OF TREATMENT	
I. C.	10^{-2}**	24 HOURS	10	10 ACTIVE	10/10
		48 "	8	2 SICK 6 ACTIVE	8/8
		72 "	9	9 SICK	7/9
		96 "	8	8 SICK (2 DAYS)	1/8
		CONTROLS	10		0/10

* 1 MG. DAILY FOR 3 DAYS

** 10^{-2} DILUTION = 513 LD_{50}

TABLE 5 shows the results obtained when mice were injected intracerebrally with graded doses of lymphogranuloma venereum virus and treated with Aureomycin by either the oral or subcutaneous routes. In this experiment, it is seen that practically complete protection was afforded to all the treated mice. However, it should be noted that the mice that received 1 mg. of drug orally or 0.1 mg. subcutaneously were sick for 3 or 4 days, whereas the mice that received 5 mg. orally or 1 mg. subcutaneously never showed any signs of illness. Furthermore, it should be mentioned that although many of the control mice survived the infection, yet practically all control mice up through the 10^{-7} dilution were seriously ill for 16 to 19 days.

TABLE 5

LYMPHOGRANULOMA VENEREUM

INOCULATION OF YOLK-SAC SUSP.		SURVIVAL RATIO OF MICE				
		TREATMENT* ONCE DAILY				CONTROLS
		ORAL DOSE		S.C. DOSE		
ROUTE	DILUTION**	5 MG.	1 MG	1 MG.	0.1 MG	
I.C	10^{-3}	5/5	5/5	5/5	4/4	1/5
	10^{-4}	5/5	5/5	3/4	5/5	1/5
	10^{-5}	5/5	5/5	4/4	4/4	3/5
	10^{-6}	5/5	4/4	4/4	5/5	3/5
	10^{-7}					2/5
	10^{-8}					3/5
	10^{-9}					5/5

* TREATMENT STARTED 24 HOURS AFTER INOCULATION AND CONTINUED FOR 5 DAYS

** 10^{-3} DILUTION = 645 LD_{50}

TABLE 6 shows the results obtained when mice were injected intravenously with approximately 4 LD_{50} of murine typhus (*R. typhi*) and treated with graded doses of Aureomycin, starting treatment 24 hours post infection and continuing treatment for 5 days. It is seen that 0.1 mg. of Aureomycin is the least amount of drug affording complete protection although 0.01 mg. of the drug protected 6 out of 10 mice.

TABLE 7 shows the results obtained when mice were injected intravenously with approximately 4 LD_{50} of murine typhus (*R. typhi*) and treated with Aureomycin by the subcutaneous route with 1 mg. per day for 5 days, starting treatment 24, 48, 72, and 96 hours post infection. It is seen that of the 20 active mice treated at 24 and 48 hours, all survived and none showed any signs of illness during the 18-day observation

TABLE 6

MURINE TYPHUS

INOCULATION OF YOLK-SAC SUSP.		TREATMENT* DAILY	SURVIVAL RATIO OF MICE	
ROUTE	DILUTION		TREATED	CONTROLS
I. V.	1:5000**	MG. 1	10/10	
		0.5	10/10	
		$\boxed{0.1}$	10/10	
		0.01	6/10	
	1:5000**			0/10
	1:10 000			0/10
	1:20 000			5/10
	1:40 000			10/10

*TREATMENT STARTED 24 HOURS AFTER INOCULATION AND CONTINUED FOR 5 DAYS

** 1:5000 = 4 LD_{50}

TABLE 7

MURINE TYPHUS

INOCULATION OF YOLK-SAC SUSP.		TREATMENT* STARTED	NO OF MICE IN GROUP		SURVIVAL RATIO
ROUTE	DILUTION		AT START	TIME OF TREATMENT	
I. V.	1:5000**	24 HOURS	10	10 ACTIVE	10/10
		48 HOURS	10	10 ACTIVE	10/10
		72 HOURS	10	10 SICK	9/10
		96 HOURS	10	6 DEAD 4 SICK	4/4
		CONTROLS	13	9 DEAD 4 SICK	0/13

* 1 MG. DAILY FOR 5 DAYS

** 1:5000 = 4 LD_{50}

period. With those treated at 96 hours, at which time 6 were dead and 4 were critically ill, all 4 treated mice survived. In contrast, 9 of 13 controls were dead at the end of 96 hours and all were dead by the 5th day. This experiment certainly demonstrates the marked beneficial therapeutic effect of Aureomycin in mice critically ill with murine typhus infection.

Table 8 shows the results obtained when mice were inoculated either intraperitoneally or intranasally with rickettsialpox (*R. akari*) and treated subcutaneously with Aureomycin for 12 days, starting 24 hours post infection. It is seen that all mice infected by the intraperitoneal route survived the infection without showing any signs of illness, regardless of whether they received 1 or 2 injections per day. In contrast, the 3 out of 10 controls that survived the infection were seriously ill for 7 to 10 days.

The mice infected intranasally and treated with Aureomycin likewise survived the infection without showing any signs of illness, whereas the 3 out of 9 controls that survived were seriously ill for 8 or more days.

Table 8

RICKETTSIALPOX

INOCULUM		TREATMENT*	SURVIVAL RATIO OF MICE	
ROUTE	DILUTION	1 MG.	TREATED	CONTROLS
I. P.	0.25 ML. YOLK-SAC SUSPENSION	DAILY	9/9	3/10
		TWICE DAILY	9/9	
I. N.	0.05 ML. AMNIOTIC SAC SUSP.	DAILY	9/9	3/9

* TREATMENT STARTED 24 HOURS AFTER INOCULATION AND CONTINUED FOR 12 DAYS

Table 9 shows the results obtained when mice were injected intraperitoneally with graded doses of yolk sacs infected with the Karp strain of scrub typhus (*R. tsutsugamushi*) and treated either orally or subcutaneously with Aureomycin, giving treatments on the 3rd, 4th, 7th, 8th, 11th, and 12th days post infection. It was found that 5 mg. of Aureomycin by the oral route completely protected all treated mice even though the infecting dose was as high as ten million lethal doses. One mg. of the drug by the oral route failed to protect any of the mice against ten million lethal doses, but protected 3 out of 5 mice against one million, and one-hundred thousand lethal doses, respectively, and completely protected 5 out of 5 mice against ten-thousand lethal doses. One mg. of Aureomycin given subcutaneously allowed 4 out of 5 mice to survive 10 million lethal doses, although all of these showed signs of mild illness for 4 days. The same amount of drug completely protected 5 out of 5 mice against one million lethal doses. In contrast, 39 of the 40 controls died from scrub typhus within 6 to 19 days post infection.

TABLE 9

SCRUB TYPHUS

INOCULATION OF YOLK-SAC SUSP.		SURVIVAL RATIO OF MICE				
		TREATED*			CONTROLS	
		ORAL		S.C.		DAY DEATHS OCCURRED
ROUTE	DILUTION	5 MG.	1 MG.	1 MG.		
I. P.	10^{-1}	5/5	0/5	4/5	0/5	6
	10^{-2}	4/4	3/5	5/5	0/5	8
	10^{-3}	5/5	3/5		0/5	11
	10^{-4}		5/5		0/5	13
	10^{-5}				0/5	15
	10^{-6}				0/5	14
	10^{-7}				1/5	14
	10^{-8}				0/5	19

*DRUG GIVEN ON 3, 4 - 7, 8 - 11 & 12 TH. DAYS AFTER INOCULATION

FIGURE 1 shows the results obtained when guinea pigs were injected intraperitoneally with graded doses of yolk-sac suspension infected with Rocky Mountain spotted fever (*R. rickettsii*), Bitterroot strain. Treatment with Aureomycin was started 48 hours post infection and consisted of 3 mg. subcutaneously once a day for 5 days. The relative temperature charts of the treated and control guinea pigs are shown in the slide. It should be pointed out that the charts represent the average temperatures of 5 guinea pigs in each of the treated groups and of 4 guinea pigs in each of the control groups. Ten out of 12 of the control guinea pigs showed scrotal swelling and 4 out of the 12 died. It is seen that complete protection against fever was afforded all treated guinea pigs against at least 2,500 infectious doses. Furthermore, none of the treated animals showed scrotal swelling or other signs of illness.

FIGURE 2 shows the results obtained when guinea pigs were injected intraperitoneally with 1 ml. of a 10 per cent brain suspension infected with epidemic typhus (*R. prowazeki*), Breinl strain. Treatment with Aureomycin was initiated on the 3rd, 4th, 5th, and 6th days post infection and consisted of 3 mg. subcutaneously once a day for 5 days. The average temperature charts of the guinea pigs in each group are shown in the chart. Groups 1 ,2, and 5 each contained 4 guinea pigs, while Groups 3 and 4 had 5 guinea pigs each. Note that if treatment was initiated before fever appeared, the febrile reaction was completely suppressed. If treatment was initiated after fever appeared, the temperature was brought

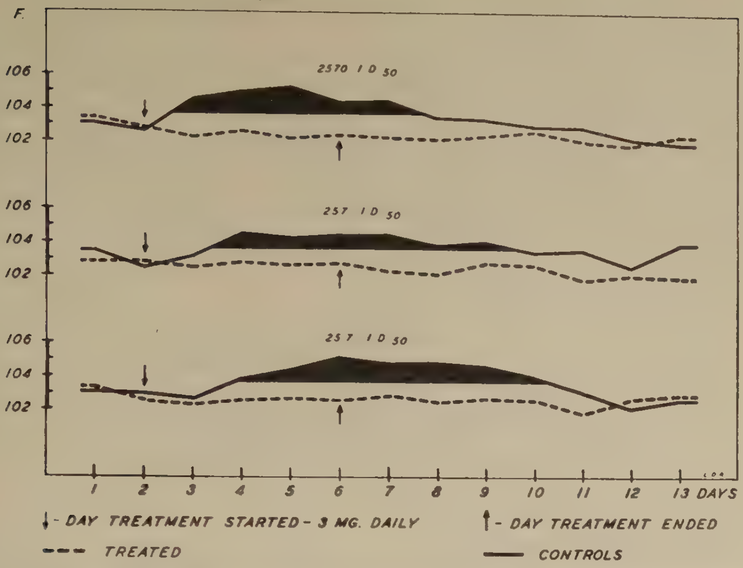

FIGURE 1. Treatment of guinea pigs inoculated intraperitoneally using yolk-sac suspension infected with Rocky Mountain spotted fever.

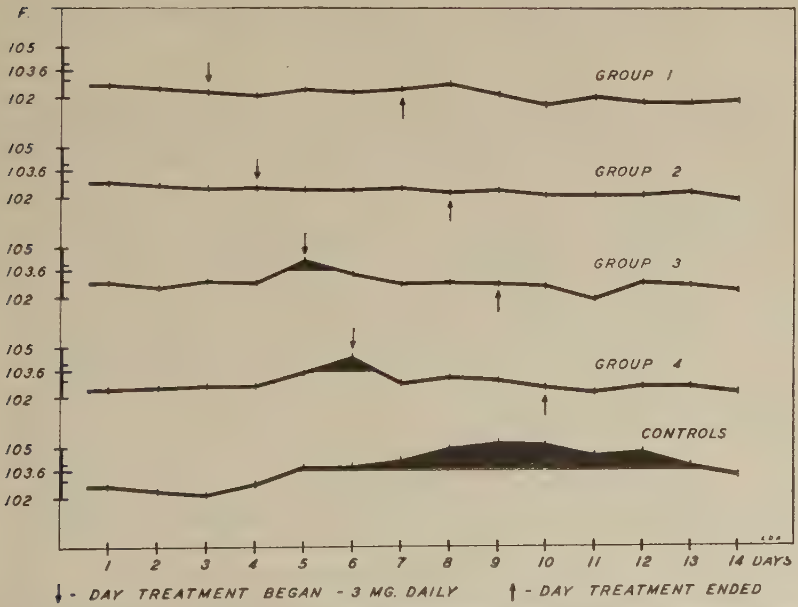

FIGURE 2. Treatment of guinea pigs inoculated intraperitoneally with 10% brain suspension infected with epidemic typhus.

back to normal within 24 to 48 hours. In contrast the control guinea pigs showed fever for an average of 7 days.

TABLE 10 shows the results obtained when guinea pigs were injected intraperitoneally with graded doses of spleen suspension infected with Q fever (*C. burnetii*), Nine Mile strain. Treatment with Aureomycin was started 24 hours post infection and consisted of 2.5 mg. subcutaneously twice a day for 8 days. It is seen that none of the 14 treated guinea pigs showed any febrile response or other signs of illness, whereas all 15 of the controls showed fever, in dilutions as high as 1:1,000,000.

TABLE 10

Q FEVER

INOCULATION OF SPLEEN SUSPEN.		TREATMENT* TWICE DAILY	GUINEA PIGS SHOWING TEMP.	
ROUTE	DILUTION	MG.	TREATED	CONTROLS
I.P.	10^{-2}	2.5	0/3	3/3
	10^{-3}		0/3	3/3
	10^{-4}		0/2	3/3
	10^{-5}		0/3	3/3
	10^{-6}		0/3	3/3

*TREATMENT STARTED 24 HOURS AFTER INOCULATION AND CONTINUED FOR 8 DAYS

FIGURE 3 shows the results obtained when guinea pigs were injected intraperitoneally with a 1:500 spleen suspension of Q fever, representing approximately 10,000 infectious doses. Treatment by the subcutaneous route with graded doses of Aureomycin was started 24 hours post infection and continued for 10 days.

The chart presents the average temperature of 4 guinea pigs in each group. The importance of the quantity of Aureomycin used in the daily dose is shown by the fact that Group 1 guinea pigs which received a daily dose of 3 mg. showed no febrile response; Group 2 which received 1 mg. daily showed low-grade fever for 48 hours; Group 3 which received 0.5 mg. daily showed the expected temperature rise but for a shortened period of time, namely 4 days; Group 4 which received 0.1 mg. daily showed no apparent difference from the control group and on the 4th day of fever the daily average dose of Aureomycin was increased to 4 mg. The temperature showed a drop to normal within 24 hours and stayed normal. The increased dosage was maintained for a total of 4 days. Controls receiving the same infectious inoculum showed continuous high-grade fever for 8 days, and controls receiving the same inoculum diluted to 1:10,000 times showed a lower temperature rise for 4 days.

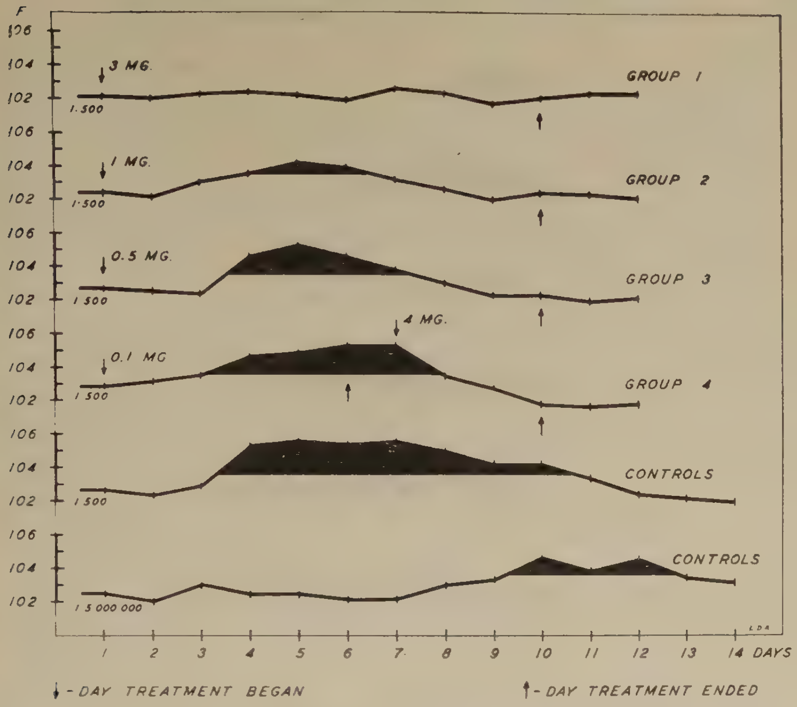

FIGURE 3. Treatment of guinea pigs inoculated intraperitoneally using spleen suspension infected with Q fever.

FIGURE 4 shows the results obtained when guinea pigs were injected intraperitoneally with a 1:500 spleen suspension of Q fever, representing approximately 10,000 infectious doses. Treatment with Aureomycin was started on the 3rd, 4th, 5th, 6th, and 7th days post infection and consisted of 3 mg. subcutaneously once a day for 3 days. The charts represent the average temperature of 4 guinea pigs in each group. It is apparent that all treated guinea pigs showed a return to normal temperature within 48 hours after the beginning of treatment, irrespective of the duration of fever prior to treatment. Essentially the same results were obtained in similar experiments carried out in guinea pigs infected with Rocky Mountain spotted fever and epidemic typhus.

In summary, from the data obtained thus far, the following general observations appear to be warranted:

1. The antibiotic Aureomycin has been shown to possess marked therapeutic activity against the viruses of the psittacosis-lymphogranuloma group and rickettsiae of the spotted fever, typhus fever, scrub typhus fever and Q fever groups in embryonated hens' eggs, mice, and guinea pigs.

2. Aureomycin shows no apparent *in vitro* activity.

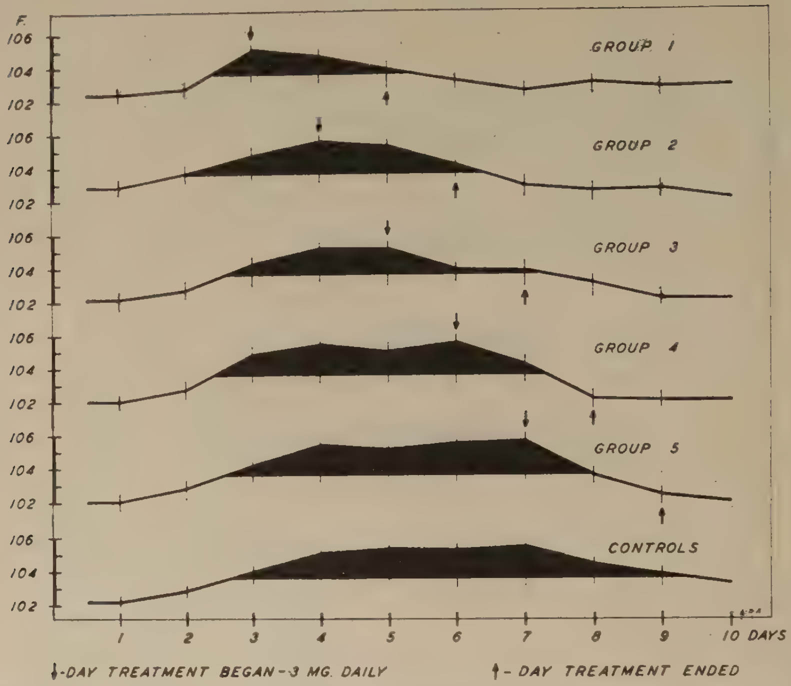

Figure 4. Treatment of guinea pigs inoculated intraperitoneally using spleen suspension infected with Q fever.

3. If guinea pigs receive a massive dose of infectious material and then are treated with Aureomycin before symptoms appear, no fever or other signs of illness develop, but antibodies in measurable degree are induced, and the animals are immune on subsequent rechallenge. On the other hand, if guinea pigs receive relatively small doses of infectious material and then are treated with Aureomycin before symptoms appear, no fever or other signs of illness develop, nor do antibodies always appear and the animals may or may not be immune on rechallenge. These findings indicate a quantitative relationship between the dosage of infecting agent and the dosage and time of administration of Aureomycin.

4. Regardless of the length of time fever has been apparent in guinea pigs infected with Rocky Mountain spotted fever, epidemic typhus or Q fever, the animals in most instances may be rendered afebrile within 48 to 72 hours by a single daily subcutaneous injection of 5 to 6 mg. of Aureomycin per kilogram body weight, for 3 to 5 days.

5. Aureomycin shows marked therapeutic activity orally, as well as parenterally, for mice infected intraperitoneally or intracerebrally with psittacosis or lymphogranuloma venereum viruses, or for mice infected intravenously or intranasally with rickettsialpox or murine typhus.

6. The question as to whether treatment with Aureomycin frees the tissues of the host from the infectious agent arises. This problem is under investigation, but the following comments may be made at the present time: Mice infected with large doses of psittacosis or lymphogranuloma venereum viruses and surviving following treatment with Aureomycin were found to harbor the infectious agent in their liver and brain, respectively, when sacrificed on the 17th day post infection. However, animals similarly infected and treated were found to be free of the infectious agent when tested on the 42nd day. In contrast, control animals infected with relatively small, nonfatal doses showed the infectious agent in their tissues at least through the 42nd day.

7. In other experiments carried out in our section, Aureomycin failed to show any therapeutic activity against the following viral infections: B strain of influenza, canine distemper, rabies, Newcastle disease, Venezuelan equine encephalomyelitis, and MEF-1 strain of poliomyelitis. Preliminary experiments with mumps virus infected chick embryos showed that Aureomycin reduced or completely inhibited the production of virus as measured by the hemagglutinating activity of the virus (allantoic fluid) for chicken red cells, but had no effect on the hemagglutinating activity of the virus *in vitro*, and apparently had no appreciable influence on the rate of multiplication and infective titer of the mumps virus as measured by infectivity tests carried out in embryonated hens' eggs.

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AUREOMYCIN—A NEW ANTIBIOTIC WITH ANTIRICKETTSIAL PROPERTIES: ITS EFFECT ON EXPERIMENTAL SPOTTED FEVER AND EPIDEMIC TYPHUS*

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THE new antibiotic recently isolated by the Lederle Laboratories Division, American Cyanamid Company, from the mold *Streptomyces aureofaciens* Duggar,[1] and named Aureomycin, has an unusual spectrum of activity covering a wide range of pathogenic organisms including rickettsiae and certain viruses.[2, 3]

The present report concerns the activity of aureomycin in two rickettsial diseases, namely, spotted fever and epidemic typhus. The observations are based on the clinical, pathological and immunological responses of the infected guinea pig under treatment with the antibiotic.

Materials and Methods

The highly virulent strain of Rocky Mountain spotted fever obtained from the Rocky Mountain Laboratory induces in guinea pigs clear-cut symptoms, characteristic pathological lesions, and a fatality rate of 100 per cent. The well known Breinl strain of epidemic typhus was supplied by Dr. J. C. Snyder of Harvard in cotton rats from which it has been established in guinea pigs. Whereas the incubation period in experimental spotted fever seldom exceeds 4 days, the typhus infected guinea pig reacts with abrupt fever after 7–8 days. In both infections, the fever runs between 40° and 41° C., usually of a continuous type over a period of 5–7 days. There is no fatality among guinea pigs due to typhus infection.

Transfers of spotted fever were made by the intraperitoneal or subcutaneous routes, injecting 1 ml. of a 10 per cent splenic emulsion derived from a frozen spleen of an infected guinea pig and suspended in skim milk. A fairly homogeneous suspension was secured by filtration through double surgical gauze. For typhus, similarly treated brain tissues served as the source of infection. Constant volumes of 1 ml. of the infectious material were used in all experiments.

Aureomycin hydrochloride was supplied by the Lederle Laboratories in two forms: vials containing 20 mg. of the golden-yellow powder, readily soluble in water for parenteral use; capsules each containing 50 mg. of the antibiotic with a small amount of impurities present. The latter form

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* This study is supported by a grant from the Lederle Laboratories Division, American Cyanamid Company

is designated for oral use only. Both modes of administration were used in the present study.

Guinea pigs of both sexes, 400–500 gm. body-weight were used and maintained prior and during the experimental period in an air-conditioned laboratory (76–78° F). Daily rectal temperatures of the animals were recorded for at least 18 days during the infection.

Experiments and Results

Following the unpublished reports of Lederle Laboratories, the general trend of the present study was to establish an efficient mode of administration of aureomycin which will protect the guinea pig from disease. This includes the minimum effective dosage, the frequency of administration, and the duration of the treatment. Emphasis was placed on the initial phase of the infection covering the entire or partial incubation period. Except for two series of experiments when the initial dose of the drug followed shortly after the infection, in all other series a delay of 24 or 48 hours in the treatment was adopted. Furthermore, attempts were made to establish the longest delay in drug administration, to counteract the disease and to inhibit febrile reactions while the disease was in progress. Consequently, clinical prophylaxis combined with curative measures were the objectives of the present study.

The activity of aureomycin in experimental rickettsial infections has been first investigated by the Lederle Laboratories. Their unpublished progress reports included spotted fever and Q fever in guinea pigs; rickettsialpox and murine typhus in mice. The tests with spotted fever showed that a daily subcutaneous injection of 4 mg. of aureomycin, 24 hours after infection and over a period of 7 days, protected guinea pigs against the infection. The total dosage in these experiments amounted to 28 mg. or 60 mg./kg. per guinea pig. Similar results were recorded in guinea pigs infected with Q fever and treated with 5 mg. daily for a total of 8 days (90 mg./kg.). The tests against murine typhus in mice injected daily for 5 days with 0.1 mg. aureomycin resulted in protection against otherwise lethal typhus infection. No untoward or toxic effects of this dosage were reported by the Lederle Laboratories.

These encouraging results, offering a wide field for further developments toward an optimal chemotherapy, stimulated us to elaborate and analyze the antirickettsial activity of this antibiotic.

Spotted Fever. Initial tests were performed on a series of 8 guinea pigs subcutaneously infected with spotted fever and treated with aureomycin by subcutaneous injections of 2 mg. daily for 7 days beginning with the day of infection (FIGURE 1). As compared with the controls showing a typical course of spotted fever followed by death, the response of all test animals was significant: 4 of them remained afebrile during 18 days' observation, 2 others reacted with moderate or abortive fevers on the 13th day, and one responded on the same day with slight fever of one-day

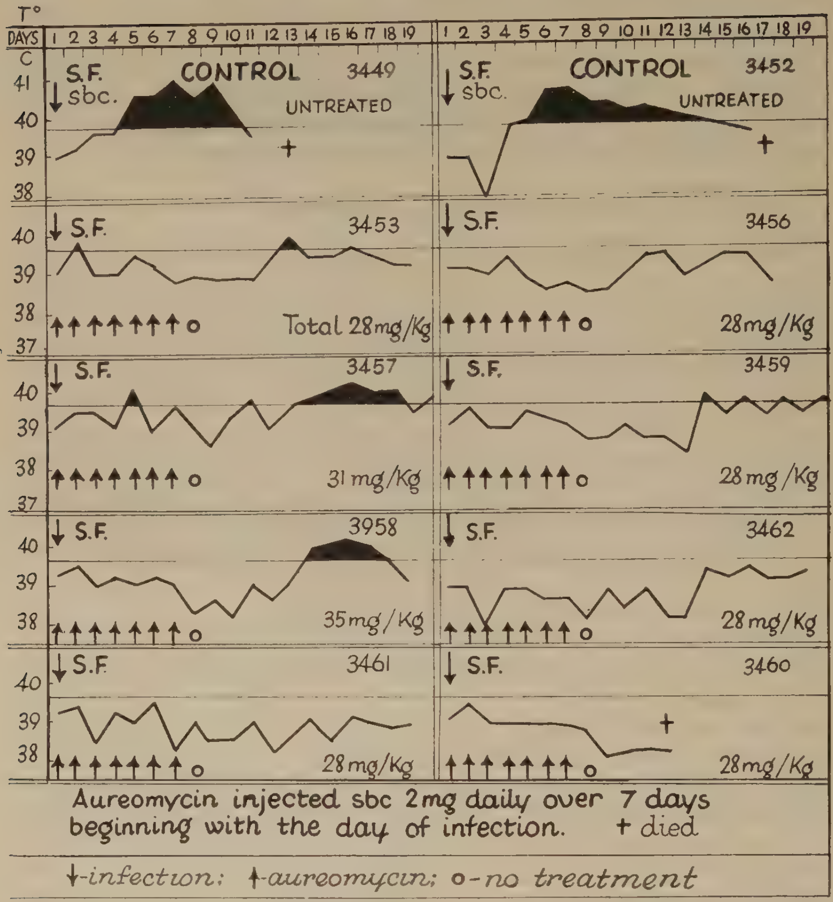
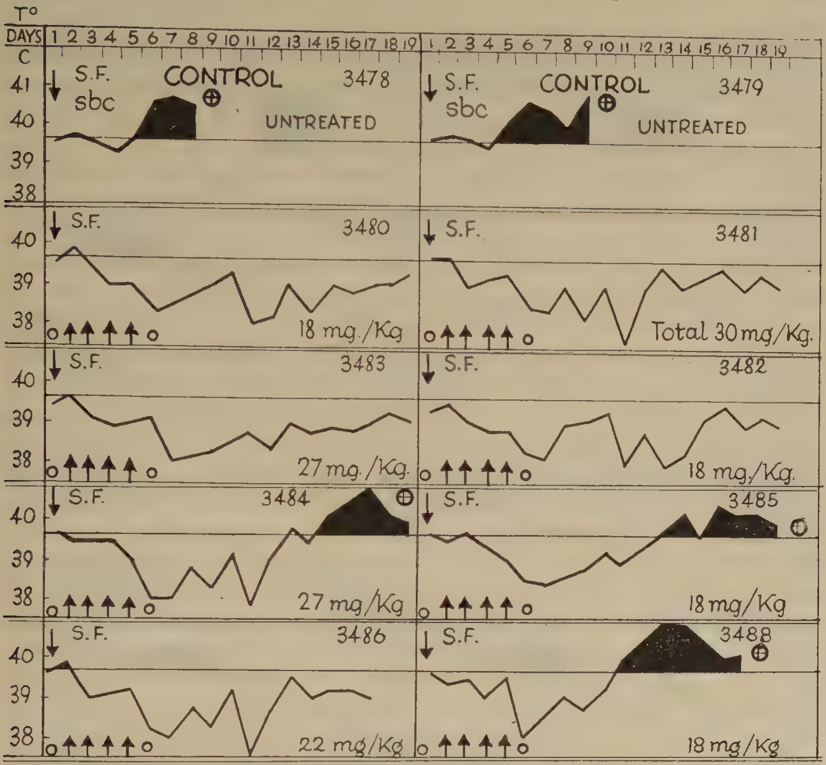


FIGURE 1. Spotted fever in guinea pigs treated with aureomycin.

duration. With the exception of one animal which died probably of diarrhea and showed hemorrhages at the site of drug injection, all other guinea pigs recovered (FIGURE 1). It was concluded from this initial series that aureomycin had a definite suppressive effect on the course of the infection in preventing the clinical symptoms or attenuating the disease after a protracted incubation.

All recovered guinea pigs of this series were reinoculated by subcutaneous route with the routine heavy dose of spotted fever, one month after the initial infection. None of the animals showed immunity.

In the next series, the administration of the drug (2 mg. daily) was initiated 24 hours after infection and continued for 4 days during incubation (FIGURE 2). Thus, the total amount of the antibiotic was reduced



Aureomycin injected sbc 2mg. daily over 4 days beginning with 2nd day of infection. ⊕-killed

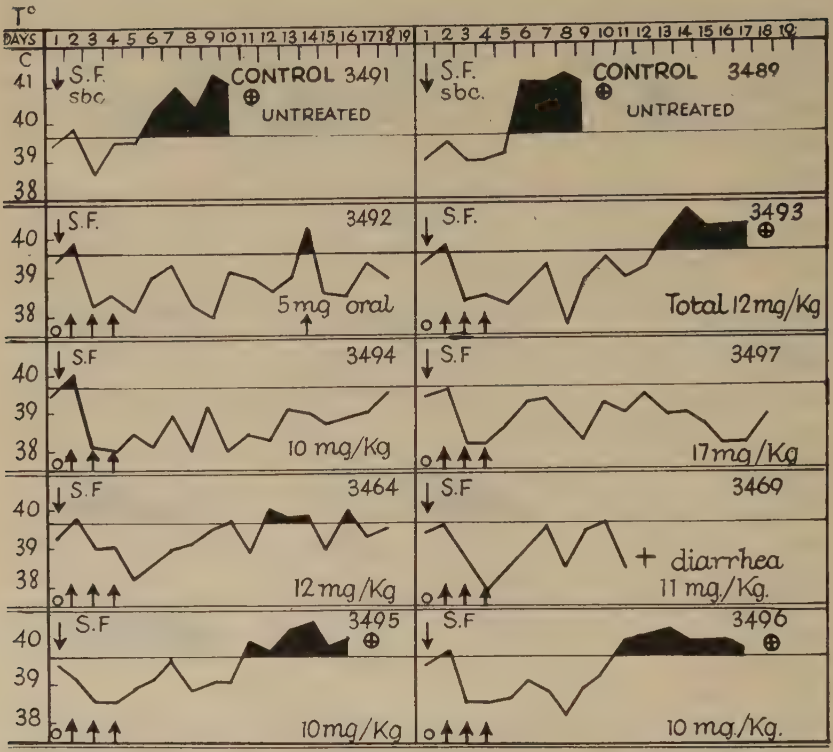
↓-Infection; ↑-Aureomycin; o-No treatment

FIGURE 2. Spotted fever in guinea pigs treated with aureomycin.

from the average of 30 mg./kg. to 22 mg./kg. Of 8 test animals, 5 remained entirely afebrile whereas 3 developed spotted fever after 10–14 days, contrary to the controls which came down with fever after the usual 4 days incubation. Consequently, despite the 24 hour delay in drug administration and the considerable shortening of the treatment, the clinical response to the infection was prevented in 62 per cent of animals in this series (FIGURE 2).

No immunity to reinoculation with spotted fever was shown by the afebrile guinea pigs.

A further reduction in dosage was made in the third series of 8 guinea pigs infected with spotted fever 24 hours prior to drug administration, and injected with 2 mg. daily for 2 days and with 1 mg. on the fourth day.



Aureomycin injected sbc 2mg daily on the 2nd & 3rd day and 1mg on the 4th day of infection.
↓ *Infection;* ↑*Aureomycin* ⊕ *Killed;* o-*No treatment*

FIGURE 3. Spotted-fever in guinea pigs treated with aureomycin.

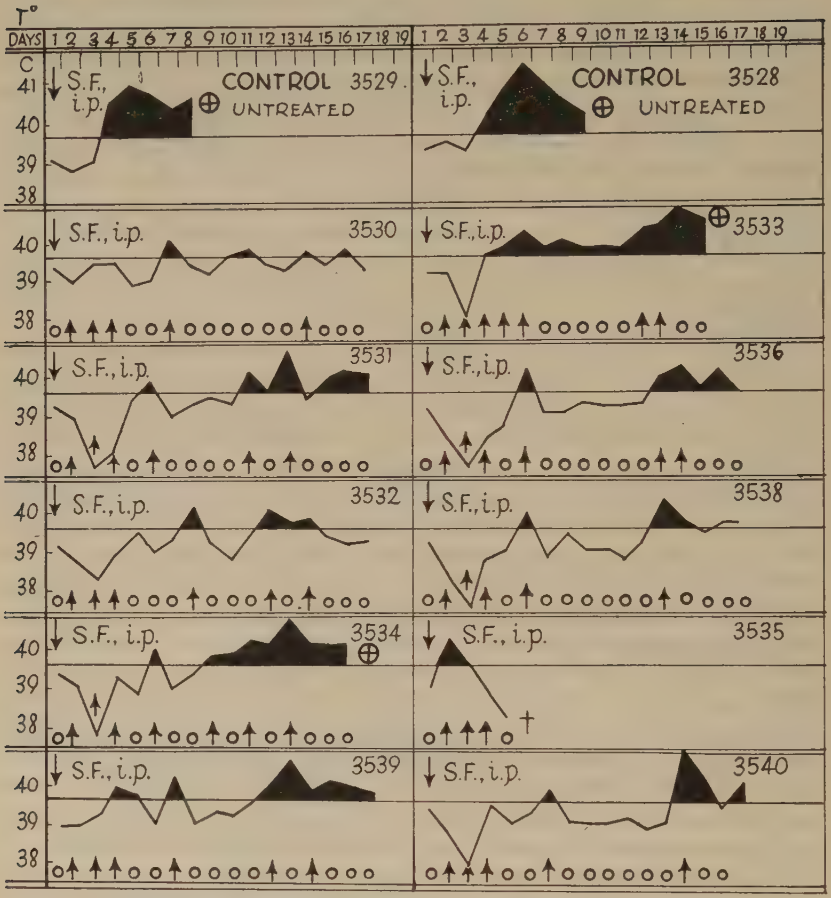
A total amount of 5 mg. per guinea pig, or an average of 12 mg./kg. were used during the experiment. As seen from charts of FIGURE 3, moderately severe fever reactions (as compared with the controls) were recorded in 3 guinea pigs after a 10–12 day incubation; two other animals remained afebrile and 2 showed abortive and slight fevers. One of them (3492) deserves attention because it was treated with 5 mg. of aureomycin orally on the 14th day after an abrupt fever attack (40.4° C.) was recorded. On the following morning, the temperature subsided as abruptly as it rose the day before.

The prompt response to a single dose of aureomycin given orally justified further exploration of this procedure. For this purpose, a small group of 4 guinea pigs (plus 2 controls) were each given 10 mg. aureomycin in aqueous solution (from capsules) orally 1 hour before the intraperitoneal infection with spotted fever. The same single dose was repeated the following day after which the treatment was discontinued. Both untreated controls came down with high fever after 4 days, whereas 2 test animals showed simultaneous slight temperature elevations on the 9th day. They were then given a single oral dose of 5 mg. aureomycin in distilled water after which the temperature was normal on the following morning. Guinea pig 3508 showing a second rise of fever was injected with 3 mg. of the drug. Another drop of temperature was recorded. The third member of the group (3511) remained afebrile until the 13th day when an abrupt rise to 40.2° C. was noted. In this case, high fever persisted despite 8 mg. of the antibiotic injected during the first 2 days of fever.

The initial treatment by the oral route was continued in a series of 10 guinea pigs with the dosage of 5 mg./kg. beginning with the second day after infection and continued for the following 2 days (FIGURE 4). As compared with the controls, which all reacted with abrupt and high fevers on the fourth day, the course of the disease in all test animals was greatly modified. First, in most of the cases, a sheer drop in body temperature to subnormal levels accompanied the intake of the antibiotic. This was followed by an almost simultaneous but moderate rise of fever on the 6th or 7th day after infection. Except for guinea pig 3533 which developed a protracted and atypical course of fever, in all 9 animals the first attack responded promptly to a single injection of 1-2 mg. aureomycin. In some animals, the temperature remained normal, despite the withdrawal of the antibiotic for 6 days, until the afebrile period was interrupted by a secondary and longer fever attack on the 10th or 14th day (FIGURE 4). Treatment was resumed if and when indicated by fevers. As compared with the prompt response of the initial symptoms, the treatment of the secondary fever attacks was as a whole less effective. Except for 2 guinea pigs which were killed, all other test animals recovered.

As to the character of the fever charts, a relapsing type can be recognized beginning with an abortive attack after a prolonged incubation period. An afebrile period of several days duration followed and was terminated by fever of varying intensity. Whether the quiescent period is due to the activities of the antibiotic *per se* or is correlated with the interaction of immune factors cannot be answered without speculation. In this regard, the significant level of aureomycin for at least 24 hours after a single injection, as demonstrated in blood of patients with lymphogranuloma venereum, should be emphasized.[3] The effect of aureomycin in our series becomes more significant considering the severe challenge of the intraperitoneal route of the infection resulting in the fulminating disease and death in the controls.

The next inquiry was to establish the longest possible delay in the protection against spotted fever by aureomycin. Since, in the great majority of the control guinea pigs, the length of the incubation period was 72 hours, it was decided to initiate treatment 48 hours after the



Initial oral administration of Aureomycin 5 mg/Kg followed by "intermittent" parenteral treatment (1-2 mg) at fever rise. ⊕ killed

† dead; ↓ infection; ↑-Aureomycin; o-no treatment

FIGURE 4. Spotted fever in guinea pigs treated with aureomycin.

infection. A series of 12 guinea pigs was infected intraperitoneally with the usual dose of splenic suspension. The untreated control guinea pigs reacted with abrupt fevers after 72 hours and ran a typical severe course of spotted fever.

To all 12 test animals, a dose of 5 mg. aureomycin in distilled water was given orally, 48 hours after infection. An "intermittent" treatment

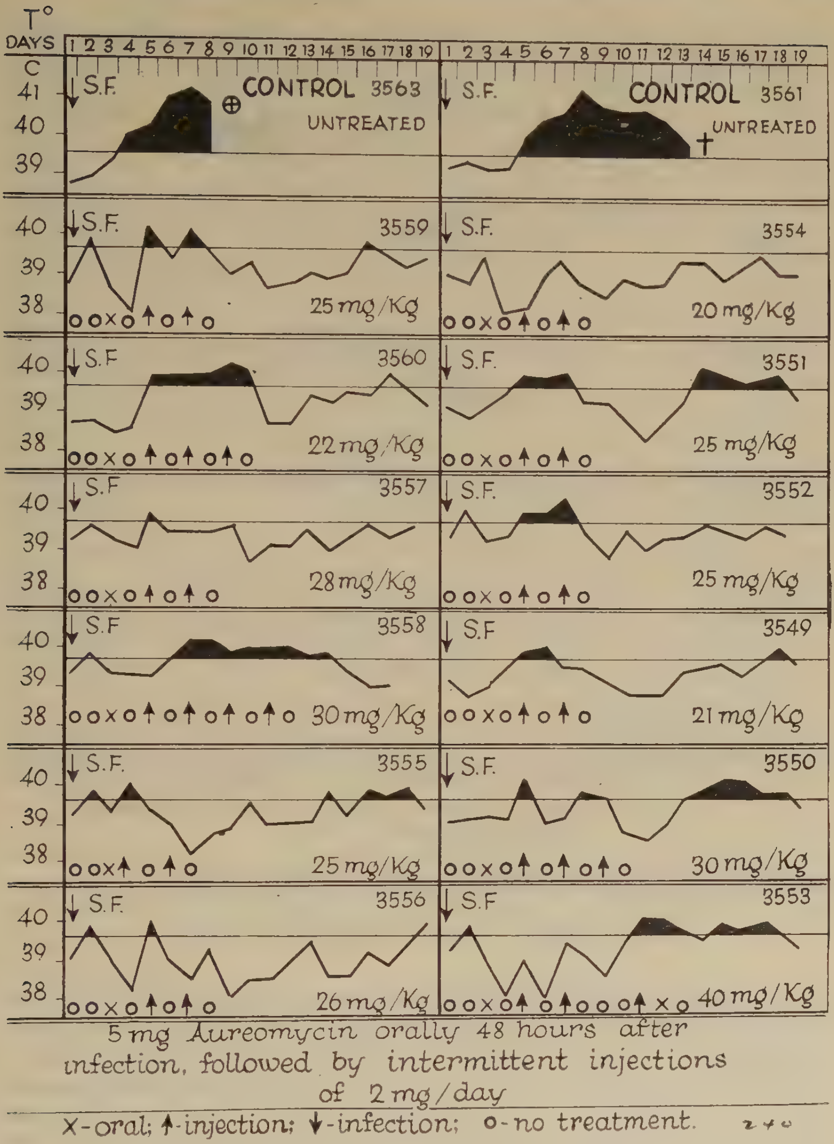


FIGURE 5. Spotted fever in guinea pigs treated with aureomycin.

was then adopted by injecting 2 mg. of aureomycin every third day (FIGURE 5).

Except for 4 animals which showed more protracted fevers and received a total of 3 or 4 intermittent injections, the remaining 8 guinea pigs of this series were injected with 2 mg. aureomycin each on the 5th

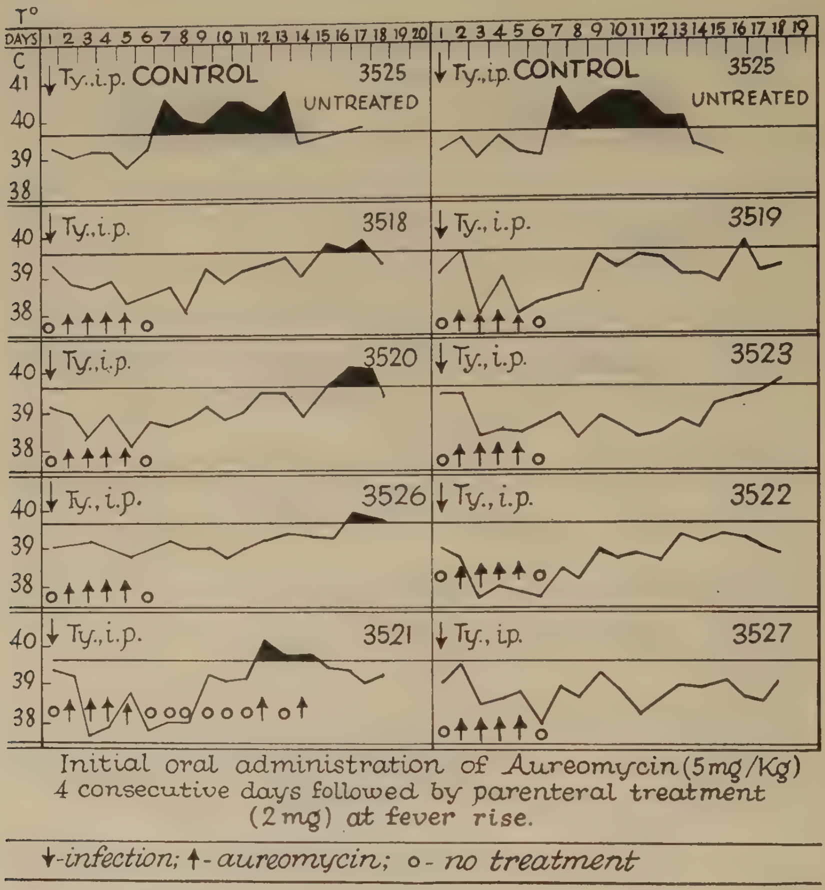


FIGURE 6. Epidemic typhus in guinea pigs treated with aureomycin.

and 7th day of infection (FIGURE 5). Apart from guinea pig 3554, which remained completely afebrile after a total of 10 mg. aureomycin, a wide variety of fever reactions was observed in the remaining test animals.

In some cases, slight temperature elevations of 1, 2 or 3 days duration at the early stages of the infection were noted, while protracted and moderate fevers were recorded in guinea pigs Nos. 3558 and 3560. Definite relapses separated from initial attacks by afebrile periods were also observed in this series. On the whole, and as compared with the controls, a significant suppression of clinical symptoms was achieved when the initial dose was given 48 hours after the infection followed by a short intermittent parenteral treatment. All treated animals recovered, gradually regaining the heavy loss in body weight (up to 25 per cent), particularly by the end of the first week.

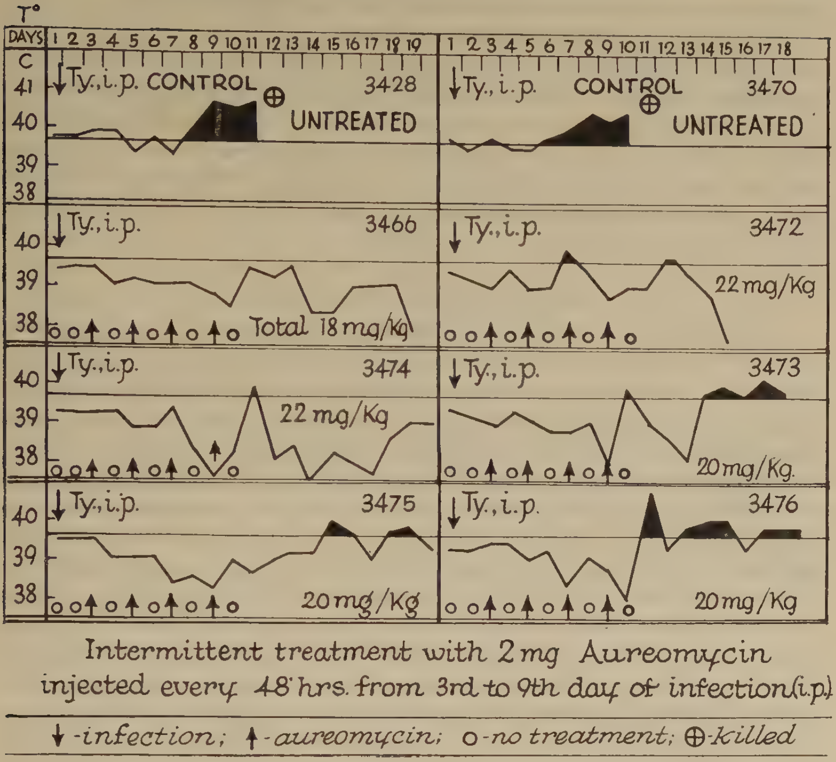


FIGURE 7. Epidemic typhus in guinea pigs treated with aureomycin.

Epidemic Typhus. The characteristic and consistent fever reactions of guinea pigs infected with the well-established strain of epidemic typhus were the criteria for evaluating the effect of aureomycin on this rickettsial infection.

In a series of 8 guinea pigs, oral administration of aureomycin was initiated 24 hours after intraperitoneal infection with typhus brain suspension (FIGURE 6). The dosage of 5 mg./kg. guinea pig per day for 4 consecutive days was given, after which the treatment was discontinued. In the 4 untreated controls, a sharp rise of fever was recorded on the 7th day, and remained on a high level for the next 7 days. In sharp contrast, 4 test animals remained afebrile during the 18-day observation, while 4 showed slight or moderate fevers on the 15th or 16th day. As a whole, typhus fever was either entirely suppressed or reduced to abortive negligible attacks after a considerably prolonged incubation.

The mode of treatment of the next series of 6 guinea pigs consisted in parenteral administration only, given at intervals every third day and beginning 48 hours after i.p. infection (FIGURE 7). Daily subcutaneous injections were given on the 3rd, 5th, 7th, and 9th day of the infection, whereupon the treatment was discontinued.

Irregular intermittent fever attacks developed in 3 guinea pigs after a protracted incubation while the other 3 guinea pigs remained afebrile.

No reports by other authors on the action of aureomycin on epidemic typhus were available. However, murine typhus in mice was investigated by the Lederle Laboratories. The results of Wong and Cox indicate that aureomycin affords complete protection to a lethal dose of murine typhus in mice.

Summary and Discussion

It is evident from the results of the present study that aureomycin is provided with potent antirickettsial properties as previously reported by the Lederle Laboratories. In their early progress report, a daily injection of 4 mg. aureomycin per guinea pig given over a period of 7 days (total 62 mg./kg.) protected the infected animals from spotted fever. In our experience, a protection against spotted fever was achieved with less than half or even with one-third of this dosage when treatment was initiated 24 or 48 hours after i.p. infection. It was also found that daily treatment for 7 consecutive days had no appreciable advantage over intermittent administration every third day with lesser amount of the antibiotic. The various modifications of the treatment still in progress seem to indicate that the initial phase of chemotherapy has a decisive influence, not only on the prolongation of the incubation period, but also on the ultimate complete or partial suppression of clinical symptoms. In the latter case, they are of the abortive type and can be checked in most cases by single injections of 1-2 mg. aureomycin. Late relapsing attacks of spotted fever are less responsive to treatment and point to a latent character of the suppressed infection. With the exception of a few guinea pigs which developed diarrhea and died, all test animals recovered, contrary to the 100-per-cent fatality rate of the untreated controls.

In typhus infected guinea pigs, still more consistent results were achieved. With the exception of a few abortive and late fever reactions, the protection in the great majority of cases was complete. This was obtained by initial oral treatment 24 hours after injection with 5 mg./kg. daily per animal for 4 consecutive days. Similar beneficial effects of aureomycin resulted from parenteral treatment beginning 48 hours after infection and then at 48-hour intervals for 4 days.

In studying the temperature curves of infected guinea pigs, attention was paid not only to the febrile reactions, but also to the afebrile periods. In the light of our previous experience with PABA, it was assumed that the effect of aureomycin is due, like PABA, to its rickettsiostatic activity resulting in the inhibition of rickettsiae and in the suppression of symptoms. A disturbance of the balance between the inhibited rickettsiae and the defensive mechanism of the infected host would then explain the flares of abortive attacks and relapses. Consequently, an immunity to the homologous rickettsiae should be afforded by the subclinical infection as was the case in PABA treated guinea pigs. However, in preliminary experiments, aureomycin-treated guinea pigs showed no immunity to reinoculation with massive infective doses of spotted fever. Furthermore, spleen of afebrile guinea pigs under antibiotic treatment when injected into normal ones induced no reactions. These results are comparable with the early findings of the Lederle Laboratories on murine typhus in mice in which, after treatment, no latent infection was found.

This intricate phase of the immune phenomena in chemotherapy deserves further investigation, which is in progress.

Addendum

While this paper was submitted for publication, the series illustrated in FIGURE 5 was reinoculated with spotted fever 4 weeks after the initial infection. Splenic suspension in 1:100 dilution was used for these tests instead of 1:10 as previously injected. Complete immunity was established in every guinea pig tested, regardless of its previous clinical responses. The immunity developed by afebrile guinea pigs is significant as it indicates a symptomless (inapparent) infection due to the suppressive, rickettsiostatic action of aureomycin.

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THE TREATMENT OF LYMPHOGRANULOMA VENEREUM AND GRANULOMA INGUINALE IN HUMANS WITH AUREOMYCIN*

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In a preliminary study which is in press,[1] we have reported results in twenty-five cases of lymphogranuloma venereum treated with Aureomycin. This study was conducted between January 22nd, 1948, and April 24th, 1948. The results obtained with this new therapeutic agent were so superior to all other forms of therapy[2] that had been used by us that we did not hesitate to continue its use. In the earlier paper, it was stated that Aureomycin was used for the first time in human beings. Apparently we were dealing with an antibiotic that could cure this virus infection in humans.

The present paper confirms our previous observations. It is a report of ten additional cases of lymphogranuloma venereum, treated with Aureomycin since our original article was sent in for publication. It also includes a follow-up report on fourteen of our original twenty-five cases, and a preliminary report on the use of Aureomycin in three cases of granuloma inguinale.

Lymphogranuloma Venereum

To date, the total number of patients to whom we have administered Aureomycin, as the sole form of specific therapy, is thirty-five, and it should be stated that they were all hospitalized. This was done so that we could be sure that the patients received an adequate amount of the drug under controlled conditions and, also, so that we could properly evaluate the results.

Most patients received 10 to 40 mg. of Aureomycin daily, by intramuscular injection. The drug was dissolved in 2 cc. of normal saline solution. In our previous series, a mild, hypochromic anemia developed when we used a special diluent. In all cases, the anemia was corrected by the administration of folic acid and iron (Folvron). In some of our earlier cases, we used folic acid and iron as an anemia preventive, while in this group we found this precaution unnecessary.

Because of a pre-existing anemia, two patients in this series received folic acid and iron while being treated with Aureomycin. We feel that the

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* We are grateful to the late Dr. Y. SubbaRow and his associates at the Lederle Laboratories Division, American Cyanamid Company, for their cooperation and for the Aureomycin used.

change from the special diluent to normal saline solution, as a vehicle, has apparently eliminated further serious consideration of anemia in relation to Aureomycin.

In the dosage used, we found that Aureomycin was non-toxic. Very few patients complained of pain at the site of injection and none had any systemic reaction. Its daily use over a three-month period of time did not produce any damage to any of the body systems; in particular, we observed no allergic skin reactions, no evidence of agranulocytosis, nor any changes in the peripheral or central nervous systems.

This use of Aureomycin is of great significance because it represents, for the first time, the high probability of a cure of a virus disease in a human being. Data bearing on this subject were presented in our original paper. The clinical results reported in this paper lend additional weight to this point. Our results were very gratifying and seemed to parallel those obtained by Wong and Cox[3] who demonstrated that Aureomycin showed remarkable therapeutic value in mice, injected intracerebrally with the virus of lymphogranuloma venereum. In the light of the evidence at hand, we feel that if Aureomycin is used in the early stages of the disease and in proper dosage, it will cure certain, if not all strains of lymphogranuloma venereum infections. Further data bearing on proper dosage and period of administration is needed.

Material. The ten new cases of lymphogranuloma venereum infection consisted of four cases of buboes, two cases of acute proctitis and four cases of rectal stricture.

The diagnosis, in each instance, was made by the usual clinical criteria —and all patients gave positive Frei reactions.

Treatment. Eight patients received 20 mg. of Aureomycin daily, by the intramuscular route. Aureomycin was used orally in two cases in the following manner: a 300 mg. dose was given every four hours—i.e., using 5 mg. per kilo of body weight.

The results obtained in these ten cases demonstrated, again, the efficacy of the drug as a specific therapy for this disease.

Lymphogranuloma Case Reports. Brief abstracts of these ten cases are as follows:

BUBO CASES:

Case 1. A twenty-four-year-old male entered the hospital, with enlarged inguinal nodes, which had been present for two weeks. On the right side, enlarged inguinal nodes measured 5 cm. in diameter. On the left, the nodes measured 2.5 cm. in diameter. There was a urethral discharge which was negative for gonococci, on smear and culture.

The patient was given 300 mg. of Aureomycin, orally, four times a day, from May 8th to May 14th. At the end of the third day of treatment, the nodes on the right side had reduced to 4 cm. in diameter. By the end of the fifth day of treatment, all tenderness had disappeared and the nodes on both sides were reduced to half their previous size. The urethral discharge had disappeared under treatment.

This patient then left the hospital, against advice, at the end of six days, and did not return to the follow-up clinic.

Case 2. A twenty-nine-year-old female entered the hospital, complaining of vaginal discharge and a swollen, tender inguinal mass of two and one-half weeks' duration. Physical examination revealed a matted, tender group of nodes in the right inguinal region which measured about 2 x 3.5 cm.

The patient was given 300 mg. of Aureomycin, orally, four times a day from April 28th to May 9th, 1948. The tenderness in the nodes disappeared in twenty-four hours and in three days the node diameter was half of its previous size. By the eighth day the mass had disappeared.

TABLE 1

FOUR NEW CASES OF BUBOES

Case No.	*Age sex*	*Days on service*	*Lesion duration*	*Diagnosis*	*Dosage*	*Results*
1	24-M	7	2 weeks	Rt. bubo 2″ diameter– Lt. bubo 1″ diameter– Urethral discharge	300 mg. Q.I.D. orally Total 8,400 mg.	After 3 days, rt. 1½″ diameter– After 5 days, rt. 1″ diameter– lt. ½″ diameter.
2	29-F	14	2½ weeks	Rt. bubo 2 x 3½ cm. Vaginal discharge	300 mg. Q.I.D. orally Total 14,400 mg.	After 1 day, node 2 x 3 cm.– After 72 hours, node 1½ cm. in diameter– After 8 days, node disappeared.
3	21-M	23	4 weeks	Lt. bubo 4 x 6 cm.	20 mg. I.M. O.D. Total 460 mg.	After 4 days, mass non-tender– 50% reduction in size
4	28-F	7	2½ weeks	Lt. bubo 5 cm. in diameter	20 mg. I.M. O.D. Total 140 mg.	After 3 days, mass non-tender– 50% reduction in size.

The patient was discharged on the eighth day of hospitalization and did not return to the follow-up clinic.

The patient received a total of 14.4 grams of Aureomycin.

Case 3. A twenty-one-year-old male entered the hospital with a history of swollen nodes in the left groin of four weeks' duration. Physical examination revealed a tender, matted mass of left inguinal nodes, measuring 4 x 6 cm.

The patient was given a daily intramuscular injection of 20 mg. of Aureomycin from June 15th, 1948, to July 8th, 1948. At the end of the fourth day of treatment, the tenderness had disappeared and the mass had shrunk to half of its previous size.

On July 17th, at the time of last examination, there was present a small area of induration 2.5 cm. in diameter, and the patient was symptom-free.

Case 4. A twenty-eight-year-old female entered the hospital, complaining of a tender mass in the left groin. Physical examination revealed a tender, matted mass of nodes in the left inguinal region 5 cm. in diameter.

The patient was given a daily injection of 20 mg. of Aureomycin from May 12th to May 19th, 1948. At the end of the third day of treatment, the mass decreased to one-half the original size and was non-tender.

After seven days of treatment the patient was so improved that she left the hospital, against advice. We have not seen her since.

(The bubo cases are tabulated in TABLE 1.)

PROCTITIS CASES:

Case 5. A thirty-four-year-old female entered the hospital on April 28th, 1948, complaining of a foul-smelling rectal discharge and rectal pain of two months' duration. Physical examination revealed a severe proctitis. On proctoscopic examination, the rectal mucosa was granular in appearance, and covered with yellowish plaques of pus. There was a thin, foul-smelling rectal discharge.

The patient was given daily injections of 20 mg. of Aureomycin from April 29th, 1948, to May 9th, 1948. Two days after the beginning of treatment, there was a marked reduction in the rectal discharge and pain. The discharge disappeared at the end of four days of treatment and, at this time, proctoscopic examination revealed normal rectal mucosa.

The patient left the hospital, greatly improved, but we have not seen her since.

Case 6. A forty-two-year-old female entered the hospital with a five-week history of rectal pain and discharge. Physical examination revealed

TABLE 2

TWO NEW CASES OF PROCTITIS

Case No.	*Age sex*	*Days on service*	*Lesion duration*	*Diagnosis*	*Dosage*	*Results*
5	39-F	19	2 months	Proctitis–with profuse rectal discharge	20 mg. I.M. Q.I.D. total 220 mg.	Marked reduction of discharge after 48 hours. Complete disappearance after 4 days. Proctoscopic examination showed normal rectal mucosa.
6	42-F	12	5 weeks	Proctitis–with rectal bleeding and pain	20 mg. I. M. Q.I.D. total 220 mg.	Rectal bleeding and pain disappeared after 7 days.

a profuse, thin, seropurulent, foul-smelling rectal discharge. She had a soft, tender, bleeding stricture, about one and one-half inches from the anal verge.

She was given a daily intramuscular injection of 20 mg. of Aureomycin from June 26th, 1948, to July 6th, 1948. After seventy-two hours, there was a marked decrease in rectal pain and discharge. On the eighth day after the onset of treatment, rectal bleeding and pain disappeared. The soft stricture was not to be felt, and proctoscopic examination was negative.

In this instance, the stricture was obviously due to mucosal and submucosal edema, which subsided under treatment. She was discharged, apparently cured.

(See TABLE 2 for these cases.)

RECTAL STRICTURE CASES:

Case 7. A forty-eight-year-old female was admitted to the hospital on April 20th, 1948, complaining of constipation. On examination, a hard, fibrous rectal stricture, which did not admit a fingertip, was palpated above the anus.

She was given 20 mg. of Aureomycin, intramuscular, for a period of sixteen days. After 320 mg. had been given, there was no change in the stricture, although the patient felt better. The constipation persisted and a colostomy was performed.

She was discharged from the hospital on June 12th, 1948, improved.

Case 8. A thirty-eight-year-old female complained of constipation and of having pencil-sized stools. She was admitted to the hospital on May 17th, 1948. On examination, she had a rectal stricture which did not admit the tip of a finger. She was observed for thirty-five days in our hospital.

She received 20 mg. of Aureomycin each day for twenty-eight days—a total dose of 560 mg. The stricture did not improve. However, the secondary infection disappeared under therapy, and on the twenty-fifth day of hospitalization she was colostomized.

Case 9. A sixty-two-year-old female entered the hospital on May 19th, 1947, complaining of severe constipation, which had been growing worse, for six years. Examination showed a rectal stricture 3 cm. above the ano-cutaneous junction. This did not admit a fingertip.

She was given Aureomycin in 20 mg. doses daily, by intramuscular injection, until she had received a total dosage of 140 mg. Under treatment, the diameter of the stool doubled and she reported that bowel movements were "easier than in years." She was subjectively improved and left the hospital, against advice, seven days later.

Case 10. A thirty-one-year-old female entered the hospital, complaining of the passage of stools per vagina, for six weeks. She gave a history of having had rectal trouble for ten years, and of a suprapubic hysterectomy, performed at another hospital, three years ago. On rectal examination, she showed a stricture that had closed down tightly and did not admit a fingertip. It was hard on palpation. Vaginal examination showed a recto-vaginal fistula, which measured about 1.5 cm. in diameter.

She received 20 mg. of the drug in daily doses, beginning June 20, 1948, until a total dose of 400 mg. had been given. There was no change in the stricture, and, as was to be expected, there was no improvement in the fistula. Obviously a colostomy was indicated in this case.

(See TABLE 3 for these four rectal stricture cases.)

TABLE 3

FOUR NEW CASES OF RECTAL STRICTURES

Case No.	*Age sex*	*Days on service*	*Lesion duration*	*Diagnosis*	*Dosage*	*Results*
7	48-F	53	Many years	Rectal stricture does not admit fingertip	20 mg. I.M. Total 320 mg.	Colostomy performed. Secondary infection improved. No improvement of fibrotic stricture.
8	38-F	35	5 years	Rectal stricture does not admit fingertip	20 mg. I.M. Total 560 mg.	Colostomy performed. Secondary infection improved. No improvement of fibrotic stricture.
9	62-F	36	10 years	Rectal stricture does not admit fingertip	20 mg. I.M. Total 140 mg.	Diameter of stools doubled in size. Home against advice. No change in stricture.
10	31-F	20	10 years	Rectal stricture Recto-vaginal fistula	20 mg. I.M. Total 300 mg.	Secondary infection improved. No change in stricture.

The results obtained in the treatment of these ten new cases, whether by the intramuscular or oral route, were similar, in every regard, to the results obtained in our previous series of twenty-five cases. The acute manifestations of lymphogranuloma venereum, namely the buboes and proctitis cases, were invariably cured. In the cases of rectal stricture, the secondary infections were cured and no extension of the lymphogranulomatous process was observed.

It is needless to point out that no antibiotic can cure a thick, fibrous rectal stricture. In no instance should surgery be withheld when indicated, whether it be colostomy or resection of the stricture. Severe mechanical conditions are the only indication for surgery. It is clear to us that Aureomycin should be used before surgery in all cases of benign fibrous stenosis of the rectum.

FOLLOW-UP RESULTS IN FOURTEEN OF TWENTY-FIVE PREVIOUSLY REPORTED CASES:

We were able to follow up fourteen of the first twenty-five cases treated which had been recorded in our first, as yet unpublished, paper on this subject. We made every effort to get the other cases to return to our follow-up clinic, but they did not do so. The follow-up period varied from two weeks to four months after discharge from the hospital and cessation of the administration of the drug. The follow-up material is comprised of two cases of buboes, one case of proctitis, and eleven cases of rectal stricture.

Two cases buboes were followed in the Out Patient Clinic. In one case, the node had been removed for study and when the patient was examined four months later, this operative wound had healed, *per primum*, and he had no complaints. The other bubo case, when examined two months later, was cured and the patient was in excellent condition. We have reason to believe that the other bubo cases had improved so much that they did not return to the follow-up clinic.

Only one proctitis case returned to the follow-up clinic. He showed a recurrence of symptoms, as evidenced by rectal discharge. In view of the fact that he is an admitted homosexual, who has been the passive partner in the practice of sodomy, it is not clear as to whether this is a true recurrence or a new infection.

Eleven cases of rectal stricture have been followed for period of time varying from two weeks to sixteen weeks. The average period of time since the discontinuance of the use of the drug was about eight weeks.

Nine patients have maintained the good results obtained under treatment. They had no rectal pain, discharge or bleeding and, where the stool diameter had increased under treatment, this increase remained. In several cases where, on initial examination, the stricture did not admit the tip of the examining finger, it did admit the examining finger at discharge from the hospital and throughout the follow-up period. All patients stated that they felt much better and considered the result good.

In one instance, there was an apparent relapse. This patient was admitted with a very soft stricture. She remained in the hospital three months and received during this time, 320 mg. of Aureomycin intramuscularly. On discharge, the stricture could not be palpated and the patient was apparently cured. One month later she was seen in our follow-up clinic, complaining of rectal pain, bleeding, and discharge. On rectal examination, no stricture was found, but proctoscopic examination showed an ulceration of the mucosa. She refused further treatment. It is therefore impossible for us to say whether this instance was a relapse or a reinfection. Certainly the stricture, which was felt on admission, was due to mucosal and submucosal inflammation, and not to scar tissue.

In one other instance, there was a recurrence of rectal discharge observed in a patient who returned to the follow-up clinic. This patient had

had a colostomy and, while in the hospital, her rectal discharge disappeared after she had received 210 mg. of Aureomycin intramuscularly. During her hospital stay she gained twenty pounds in weight. Her discharge recurred, subsequent to leaving the hospital, but the patient refused further treatment.

In general, it may be stated that most of our rectal stricture cases gained weight, even without the benefit of colostomy, and this gain in weight was maintained during the follow-up period.

In all of these cases, Aureomycin not only cleared up the secondary infection, but not in any single case was there seen an extension of the disease process clinically, in so far as we could determine. It is still too early to draw final conclusions as to the effect of Aureomycin on the extension of the disease process.

Granuloma Inguinale

In view of the unusual therapeutic range of Aureomycin and its low toxicity, it seemed desirable to use this antibiotic in patients suffering from granuloma inguinale. Other types of therapy which have been used in this disease—chemotherapy, tartar emetic, Fuadin (Winthrop), Anthomaline (Merck), radiation therapy and podophyllin in olive oil—were unsatisfactory because of the frequency of relapses and the chemoresistance of the chronic cases. We felt that Aureomycin might be effective but less toxic than streptomycin in this condition.

Three cases of granuloma inguinale have been treated by us with Aureomycin with eminently satisfactory results. This is the first use of Aureomycin to our knowledge for the treatment of this disease in human beings.

The abstracts of the case histories are as follows:

Case 1. The patient is a thirty-nine-year-old white seaman who entered the hospital on April 1st, 1948, complaining of an ulcer of the foreskin of the penis. He gave the following history:

The ulcer had appeared four months previously in January of 1948. At first he treated it with sulfathiazole ointment, soap and iodine. He saw a doctor on January 12th, 1948, who treated him with one of the sulfa drugs for six days. At that time, the Frei and Wassermann tests were repeatedly negative. His condition did not improve and the patient, therefore, entered a hospital in Galveston, Texas. There, blood and spinal fluid tests were negative. He was treated with penicillin therapy, but the ulcer did not respond.

On March 8th, 1948, he entered a Staten Island hospital. A report from this institution stated that darkfield examinations for *Treponema pallida* were negative. Blood and spinal fluid tests for syphilis, the Frei test for lymphogranuloma venereum, and smears for the Ducrey bacillus were all negative. The patient refused biopsy of the lesion. At this time, he gave the additional history of a gonorrheal infection and a penile lesion for a period of three months in 1927, for which he had received arsenic and mercury treatment. Because it was considered that this treatment was probably inadequate, the patient was given 40,000 units of penicillin, every two hours, for eighty-five doses. He left the hospital, against advice, because the ulcer did not improve.

Examination at Harlem Hospital showed no abnormal findings except a tender ulcer (see FIGURE 1), which measured 4 x 6 cm. Biopsy was done on April 7th, 1948, and Donovan bodies were found.

On April 10th, 1948, 20 mg. of Aureomycin was administered by the intramuscular route and given daily thereafter. Two days after treatment was initiated, there was marked improvement, the tenderness had disappeared, and the patient felt better. On the third day of treatment, the base of the ulcer was dry and it was greatly reduced in size. Six days later, there was no further discharge from the ulcer and the lesion was one-quarter of its previous size.

On April 24th, 1948, the ulcer measured ½ x 1 cm. and the dose of Aureomycin was increased to 20 mg. twice a day. On May 8th, 1948, the base of the lesion was indurated, although still smaller in size. It was non-tender and there was no discharge. The patient had received, by this time, 1.06 grams of Aureomycin, with no toxic reactions.

On June 4th, 1948, after 2.02 grams of Aureomycin had been administered, the ulcer was completely healed.

The patient was discharged on June 7th, 1948 (see FIGURE 2). He has been followed since, but there is no sign of recurrence.

Case 2. The patient, a thirty-two-year-old female, was admitted with a history of known granuloma inguinale for the past seven years. At its inception, she had experienced itching around the vagina and, two days later, an eruption appeared, which spread rapidly to the perineal and perirectal regions.

In 1942, a diagnosis of granuloma inguinale was made in our Out Patient Department. The lesion had healed under Fuadin treatment, but two weeks after cessation of treatment, it recurred. She has had numerous courses of Fuadin since, only to have the pathology recur at the conclusion of treatment.

Three months before the present admission, the patient developed an eruption in the skin of the pudendal and perineal region. She could not sit down because of the pain, so therefore she remained in bed or stood up most of the time.

On admission there was profuse, foul-smelling discharge from these granulating and ulcerating areas. In fact, the stench was so foul that, when her dress was raised for examination, the odor permeated a large

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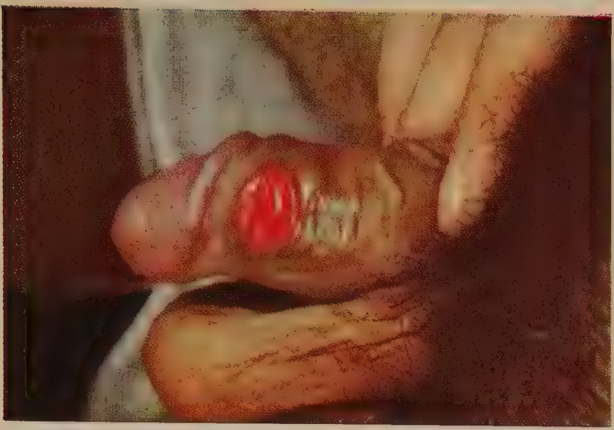
FIGURES 1–4 (*see opposite page*)

FIGURE 1. (Case 1) Ulcer of penis due to granuloma inguinale—before treatment.

FIGURE 2. (Case 1) Healed ulcer of penis after treatment.

FIGURE 3. (Case 2) Granuloma inguinale—Healed ulceration of perineum and vulva, posterior view—after treatment.

FIGURE 4. (Case 3) Granuloma Inguinale—View of healed ulcer of vulva—after treatment.



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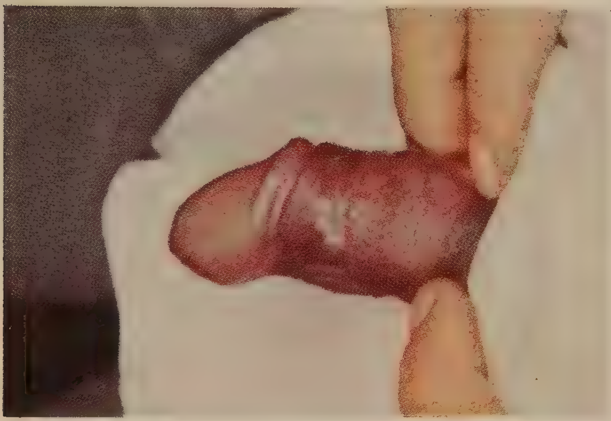

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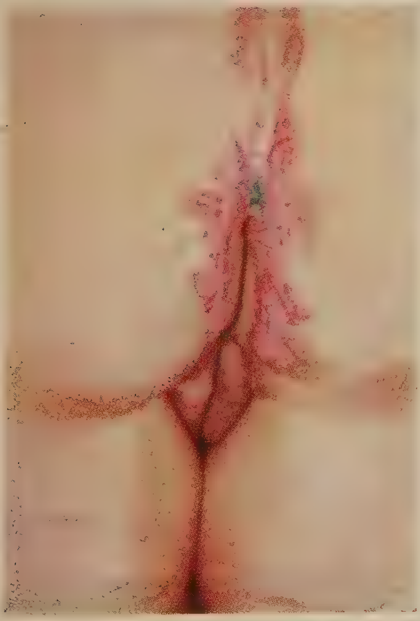

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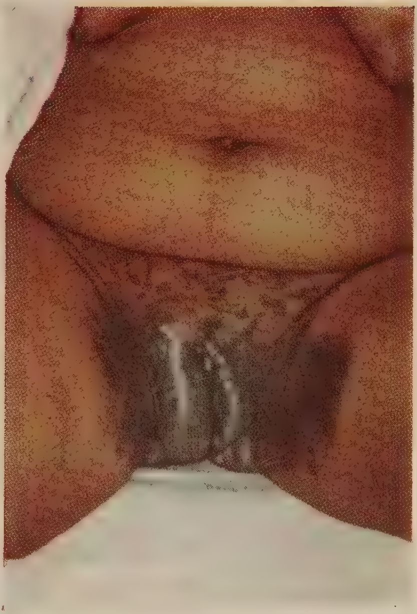

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room. Laboratory studies revealed a negative Frei test and a positive Kahn. Other laboratory findings were within normal limits. Biopsy, on this admission, showed the presence of Donovan bodies in a giemsa stain.

On May 25th, 1948, the patient was started on oral Aureomycin—300 mg. every four hours. After four days she had no toxic reaction. At the end of six days of treatment the purulent discharge had ceased, and she felt subjectively better, being free of pain. The lesion was reduced about ten per cent in size. After three weeks of treatment, the lesion was seventy-five per cent healed.

The patient received a total dosage of 75.6 grams and was discharged completely healed, and with a sense of well-being (see FIGURE 3).

Case 3. A fifty-three-year-old female was admitted to Harlem Hospital with a history of perirectal swelling and discharge of ten years' duration. She had had two previous operations for removal of granulation tissue and a perianal fistula. Two years prior to the present admission to the hospital, the left labia became so swollen and tender that she could not walk, except with extreme pain. There was a foul-smelling discharge present. Examination was negative, except for pathological findings confined to the genitalia. The left labia were enlarged to three times their normal size and exuded a foul-smelling discharge. There was a thick, exquisitely tender, perirectal mass. Laboratory studies revealed a positive Frei test and the Wassermann reaction was negative. Other laboratory findings showed normal values. Biopsy was done and revealed the presence of Donovan bodies.

The patient was given 20 mg. of Aureomycin daily for twenty-eight days, receiving a total dosage of 560 mg. After eight days of treatment, the patient was free of pain and there was a moderate reduction in the edema and in the foul discharge (see FIGURE 4).

At the time the patient left the hospital, the discharge had completely disappeared and her vulva had returned to normal.

CLINICAL RESULTS:

The rapid and satisfactory response of these three proven cases of granuloma inguinale to Aureomycin seemed to indicate that it has a definite place in the treatment of this condition. Our follow-up has not been sufficiently long for us to comment upon the possibility of permanence of the cure. The good results, thus far obtained, demonstrate the necessity of further study of Aureomycin, particularly because it is non-toxic and can be taken orally (TABLE 4).

Oral Aureomycin. We have given Aureomycin, by the oral route, in two cases of lymphogranulomatous buboes and in one case of granuloma inguinale. The results were satisfactory and comparable to those obtained by intramuscular injections. The dosage was computed at 5 mg. per kilo of body weight. This dose was given every four hours. One patient received 8.4 grams and one other received 14.4 grams, while a third received 75.6 grams. No toxic reactions of any type were observed.

Table 4

Three Cases of Granuloma Inguinale Treated with Aureomycin

Case No.	*Age sex*	*Days on service*	*Lesion duration*	*Diagnosis*	*Dosage*	*Results*
1	39-M	38	4 months	Granuloma inguinale, penile ulcer	20 mg. I.M. Then 40 mg. I.M. Total 2,020 mg.	Cured
2	32-F	43	7 years	Granuloma inguinale, vulvo-perineal	300 mg. Q.4.H. Total 75,600 mg.	Ulceration healed
3	53-F	28	10 years	Granuloma inguinale, vulvo-perioneal	20 mg. I.M. Total 560 mg.	Ulceration healed Edema of vulva reduced

Table 5

Case 2—Oral Aureomycin 300 mg.

		Tube dilution						*Activity (micrograms/ml.)*
		1	*2*	*4*	*8*	*16*	*32*	
Before drug	Undilute	++	++	++	++	++	++	<0.05
1 hour after 300 mg. orally	Undilute	−	−	−	−	−	+	
	1——10	−	−	+	++	++	++	1.0
2 hours	Undilute	−	−	−	−	−	−	
	1——10	−	−	−	+	++	++	2.0
3 hours	Undilute	−	−	−	−	−	+	
	1——10	−	−	+	++	++	++	1.0
4 hours	Undilute	−	−	−	−	−	+	
	1——10	−	−	+	++	++	++	1.0
300 mg. orally 1 hour after second dose	Undilute	−	−	−	−	−	+	
	1——10	−	−	+	+	++	++	1.0
2 hours after second dose	Undilute	−	−	−	−	−	−	
	1——10	−	−	−	+	++	++	2.0

Blood Levels. A sampling of the blood levels of Aureomycin was done on two patients, one on oral therapy and the other on intramuscular therapy. The method used in carrying out this work on blood levels was developed by Dornbush[4] and was done in his laboratory.

In micrograms of activity, per milliliter, the highest blood level, namely two micrograms per milliliter, was obtained two hours after oral administration of 300 mg. of Aureomycin, as shown in TABLE 5.

In the other patient, who received 20 mg. intramuscularly, the highest concentration of the drug, namely one microgram per milliliter, was noted five hours after injection of the drug, as shown in TABLE 6.

TABLE 6

CASE 3—20 MG. by I.M.

		Tube dilution						Activity (micrograms/ml.)
		1	*2*	*4*	*8*	*16*	*32*	
Before dose	Undilute							
24 hours after last dose	Undilute	++	++	++	++	++	++	<0.05
1 hour	3——10	–	–	+	++	++	++	0.33
2 hours	Undilute	–	–	–	–	+	++	
	1——10	–	+	++	++	++	++	0.5
3 hours	Undilute	–	–	–	–	+	++	
	1——10	–	+	++	++	++	++	0.5
4 hours	Undilute	–	–	–	–	+	++	
	1——10	–	+	++	++	++	++	0.5
5 hours	Undilute	–	–	–	–	–	+	
	1——10	–	–	+	++	++	++	1.0
6 hours	Undilute	–	–	–	–	–	+	
	1——10	–	–	+	++	++	++	1.0

No true comparison can be made because of the relatively massive dose given orally in contrast to the small dose given intramuscularly.

Summary

1. Ten new cases of lymphogranuloma venereum treated with Aureomycin are reported, in detail. Together with the twenty-five cases reported in a preliminary paper, this makes a total of thirty-five cases. The results were excellent and we believe that this antibiotic is a superior, specific form of therapy for the lymphogranuloma venereum virus infection. This antibiotic is also very effective against the secondary bacterial invaders present in this disease.
2. Fourteen of the original twenty-five cases reported in our, as yet unpublished, paper have been followed for periods of time varying from two to sixteen weeks after discharge from the hospital. These cases show that the curative effects of Aureomycin persist after treatment has been stopped.
3. Ulcerative lesions in three patients with proved granuloma inguinale were healed by the use of Aureomycin. Its lack of toxicity, in the dosage used, and also the fact that it can be taken orally, demand further study of this antibiotic in this disease.

Conclusions

Aureomycin is the treatment of choice in all cases of lymphogranuloma venereum infection. If mechanical conditions demand, surgery, in conjunction with Aureomycin, is indicated.

Aureomycin will heal ulcerations produced by the causative agent of granuloma inguinale. Further extensive clinical research in the use of Aureomycin in the treatment of granuloma inguinale is warranted.

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TREATMENT OF Q FEVER IN MAN WITH AUREOMYCIN*

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An opportunity to evaluate aureomycin therapy, found by Wong and Cox[1] to be effective in experimental Q fever, was afforded by the occurrence of human cases of Q fever in southern[2] and northern California.[3] In the northern part of the state, where this study was conducted, cases of the disease were first recognized in considerable numbers early in 1948[3] and to the time of writing more than 125 cases have occurred.

Studies with aureomycin were undertaken in the middle of May, 1948, and thus far, 23 patients have been treated. The preliminary results of this study form the basis of the present report.

Plan of Study

It was considered impractical, for a number of reasons, to treat alternate patients with aureomycin. The relatively small number of patients available at any one time, the large geographic area over which they were scattered, and the lack of knowledge of desirable doses of the drug by the parenteral and oral routes were the determining factors in this decision. It was believed that evidence for the effectiveness, or lack of effectiveness, of aureomycin might be obtained more rapidly by selecting for treatment a group of patients whose course was such that a severe, prolonged illness appeared likely. In the event that the illness terminated in each instance within a relatively short interval, a case for the effectiveness of the drug in Q fever could be established. Such a procedure obviously necessitated a comparative study of numerous untreated individuals in order to obtain as complete a spectrum as possible of the clinical picture. Through the cooperation of physicians in three different areas where cases were occurring, pertinent clinical information was eventually accumulated on a total of 90 patients with a serological diagnosis of Q fever, and when collated gave some conception of the type of illness being encountered in different age groups in the 3 localities. Patients to be treated were chosen in the older age groups (*vide infra*) in each of the 3 areas, and the clinical diagnosis in all instances was checked by complement-fixation tests for Q fever.

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The authors also wish to thank Mrs. Beatrice England, Mrs. Jeane Harris, and Mrs. Florence Jensen for technical assistance in the serological studies and Mr Oscar Brunetti and Miss Margery Maggs for technical assistance in the animal work.

The aureomycin was supplied by Dr. Herald R. Cox.

The duration of fever was selected as the best objective criterion for the evaluation of the therapeutic effect of the drug, and in this connection several points on the febrile course in untreated patients should be mentioned. During the early phase of illness, the temperature was usually high, often exceeding 104° F. In certain instances, the fever terminated within a few days, while in others, the initial high febrile phase was followed by a prolonged period of low grade fever. These differences were, in major part, associated with age, as will be brought out below.

The use of the duration of fever as the sole criterion of the patient's response to therapy has obvious limitations, since the marked symptomatic improvement which may occur before the fever has completely subsided is not reflected in the temperature graph.

Course in Untreated Patients

TABLE 1 shows the duration, by age groups, of the febrile course in the 90 patients comprising the untreated group. With due allowance for the fact that the individuals with a severe illness were more likely to seek medical aid, and thus weight the group in favor of the more severe course, it is still apparent that Q fever as seen in central and northern California has frequently been a disease with a protracted course, especially in older individuals. The short duration of the disease in the younger age groups is similar to that reported in military groups, and contrasts sharply with the course in older individuals.

TABLE 1

DURATION OF FEBRILE COURSE IN UNTREATED PATIENTS, ACCORDING TO AGE

Age group	*Number of febrile days**			*Total number patients*
	7 or less	*8–14*	*15 or more*	
25 or less	38	10	3	51
26–35	11	6	5	22
36 or more	3	2	12	17
Total	52	18	20	90

* Febrile day defined as one on which one or more oral temperature readings were 99.2° F. or higher.

It was early apparent that in young individuals, *i.e.*, those under 25 years of age, the course of the disease was subject to such extreme variation, and was so frequently short, that comparative studies of treated with untreated patients would present great difficulties. Among individuals 26 or older, on the other hand, such a large proportion had an illness of considerable severity and long duration, that this age group appeared to offer the best opportunity for comparative studies, and consequently patients to be treated were selected, with certain exceptions noted below, from this older age group.

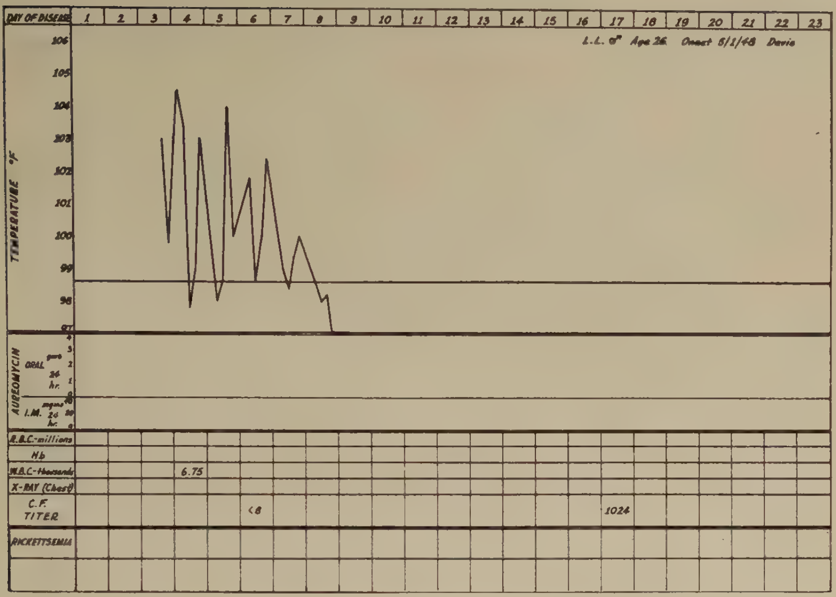

FIGURE 1. A mild case of Q fever (untreated).

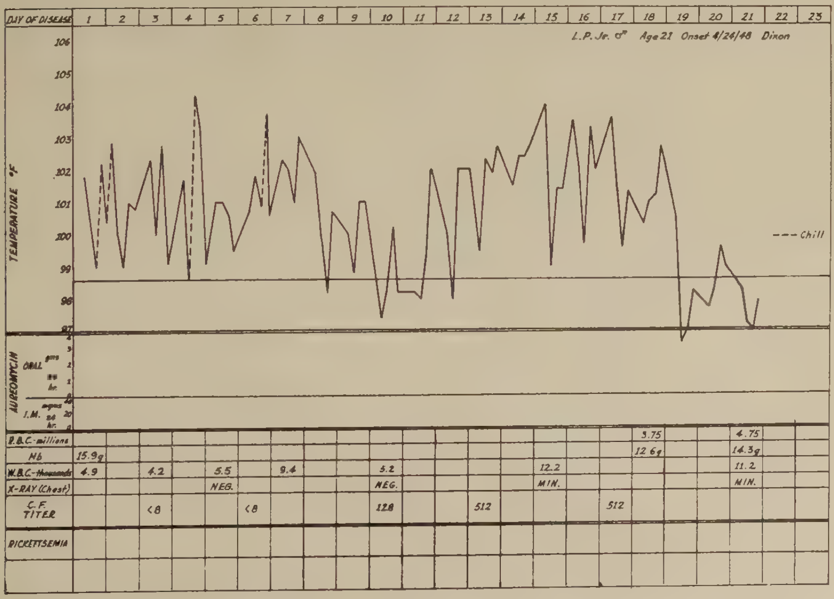

FIGURE 2. A moderately severe case of Q fever (untreated).

Figures 1 and 2 present data on a mild and on a moderately severe case, respectively, of Q fever which did not receive aureomycin. Like most of the patients in the "untreated" group, they received penicillin or a sulfonamide drug.

Course in Treated Patients

The patients to whom aureomycin was given were chosen in the light of experience with the untreated group in an effort to pick those individuals most likely to have a long and stormy course. As mentioned above, these individuals were primarily in the older age groups, and since the disease was being studied in 3 different areas, the treated patients were compared with untreated persons of the same age group in the same area in order to obviate, insofar as possible, unknown factors associated with geography, exposure to infection, mode of transmission, differences in infecting strains, etc.

With the exception of two younger patients, selected because they were unusually ill, all were over 25 years of age and the majority were over 30 years of age. All but one (J. R., table 2) had been ill for 4 or more days, and all were becoming increasingly ill rather than showing improvement. All had had one or more true rigors and, except for 1 individual (R. S., table 2), had had oral temperature readings of 104° F. or more.

Table 2

Results in Patients Treated During Acute Phase with Oral Aureomycin

					Febrile days		
No.	*Patient*	*Age*	*Max. temp.*	*Chills*	*Before treatment*	*After treatment*	*Total*
1.	J. R.	22	104.4	+	2	2	4
2.	H. T.	22	104.4	+	4	3	7
3.	G. N.	28	104	+	4	3	7
4.	L. T.	48	104	+	4	3	7
5.	J. P.	27	105	+	5	0	5
6.	G. S.	63	104.4	+	6	4*	10
7.	C. G.	48	104	+	7	5	12
8.	F. M.	65	104	+	8	2	10
9.	R. J.	38	104	+	8	3*	11
10.	R. S.	28	103	+	9	3	12

* Relapse following cessation of therapy, see text.

Of the 23 patients treated to date, 18 received aureomycin within 12 days of the onset of illness. In this group of 18, 2 received intramuscular therapy only, 2 intramuscular, followed by oral therapy and 14 oral therapy only. The remaining 5 patients, who had been febrile for from 23 to 77 days after onset, were treated orally.

In the following discussion, data are presented only on those patients, 14 in number, who were treated early in the disease and on whom etiological studies confirming the clinical diagnosis have been completed.

All of these have been followed for more than two weeks after cessation of therapy.

Intramuscular Therapy.—The first 4 patients were treated with 40 mg. of aureomycin hydrochloride daily given in two injections of 20 mg., each at 12-hour intervals. Two patients (R. B. and J. C.) showed prompt symptomatic improvement with fall of temperature to normal within 72 hours. A third (A. B.) also improved, but continued to show daily low elevations of temperature until placed on oral therapy. The fourth patient (D. D.) showed no improvement during 4 days on this regime; he was then put on oral therapy and his temperature fell to normal within 48 hours. Severe pain at the site of injection, even when phosphate buffer (pH 7.2) was used as the diluent, was the cause for discontinuing parenteral therapy in the latter two patients.

The results obtained with these small intramuscular injections are not considered convincing.

Oral Therapy.—Eight patients over 25 years of age, with a spread from 28 to 65 years, and 2 unusually ill patients under 25 were treated with aureomycin by the oral route.

These individuals received during the first 24 hours a dose of 3.2 or 4.0 g. of the drug, and were then maintained on 1.6 or 2.0 g. per day for 4 or more days. The smallest total dose of drug administered was 8.0 g., the largest 27.5 g.

In every instance, symptomatic improvement, manifested best by the return of appetite, was noted within 48 hours, and a considerable decline of temperature occurred within 48 to 72 hours after therapy was commenced. The temperatures of 8 patients fell to within normal limits (99° F. or below) during the first 3 days of therapy and the temperatures of the other 2 became normal after 4 and 5 days of therapy. The data on these 10 patients are summarized in TABLE 2.

The drug was discontinued in some instances within 24 hours after the temperature became normal. In 8 of the 10 patients presented in TABLE 2, convalescence was uneventful. In the remaining 2 (G. S. and R. J.) who had received only 10.0 g. of the drug over a 4 day period, fever recurred 2 days after the drug had been stopped. Therapy was re-instituted and the temperature returned to normal in much the same pattern as had been observed during the first episode.

The course of the illness in 5 patients is illustrated in FIGURES 3–7, and a brief description of each of these patients follows.

Case 3 (FIGURE 3). G. N., male, 28, a student, became ill on May 24, 1948, and was admitted to the infirmary, that evening, with complaints of headache, malaise, and feverishness. During the next four days his temperature fluctuated repeatedly from normal to peaks of 104° and he had repeated shaking chills. Physical examination, at this time, did not reveal any abnormal findings in chest or abdomen. The white blood cell count was 4600.

Oral aureomycin therapy was begun on May 27, 3.6 g. being given

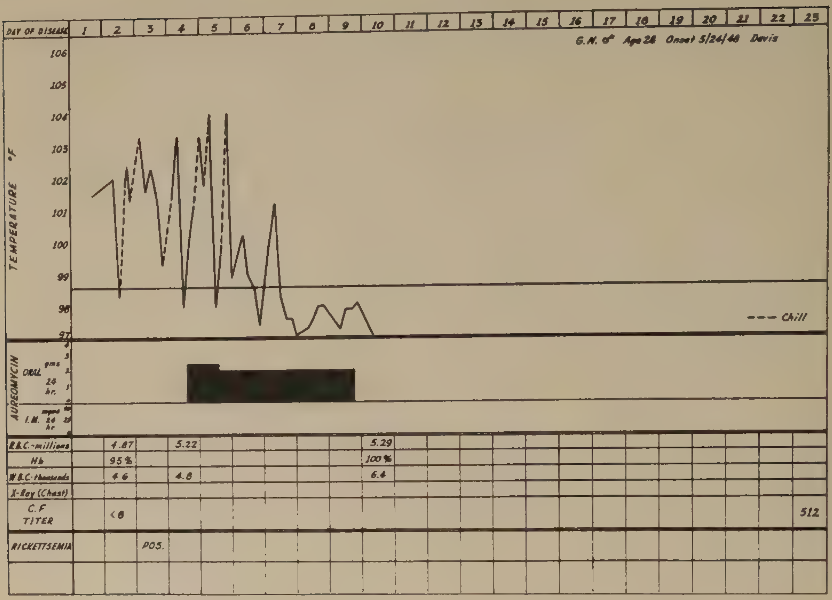

Figure 3. Course in patient treated with aureomycin (Case 3).

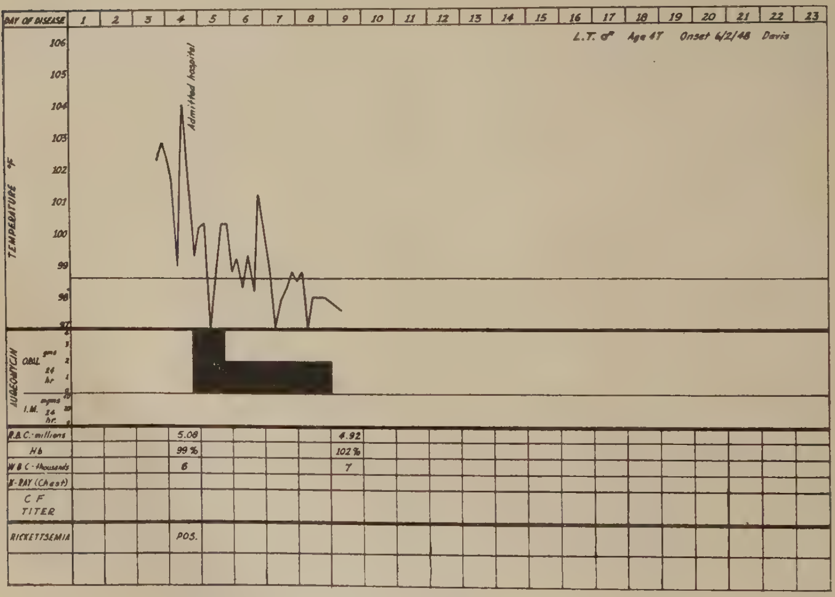

Figure 4. Course in patient treated with aureomycin (Case 4).

during the first 24 hours and 1.8 g. daily thereafter for 4 days, a total of 10.8 g. On May 28, the patient again had a chill and his temperature rose to 104° F. At this time, he appeared acutely ill, the liver was palpable 1 fingerbreadth below the costal margin and was slightly tender, and the spleen was felt 1 fingerbreadth below the costal margin. The following day, he felt considerably better. His temperature returned to within normal limits on May 31. For several days following discharge on June 2, the patient complained of weakness and sweating, but his temperature remained normal.

Etiological data: Inoculation into guinea pigs of blood taken on May 26 showed the presence of *Coxiella burneti.* In addition, complement-fixing antibodies to *Coxiella burneti* appeared in the patient's blood, the titer rising from <1:8 on May 25 to 1:512 on June 15.

Case 4 (FIGURE 4). L. T., male, 47, a plumber, became ill on June 2, 1948, with complaints of malaise, headache, feverishness, chilliness, pain in the eyeballs, and generalized aching. He was admitted to the hospital on June 25 following a shaking chill which lasted from 10 a.m. to noon. On physical examination at this time, the patient appeared fatigued, the skin was wet and hot. No abnormal findings were discovered on examination of the heart, lungs, and abdomen. The white blood cell count was 6,000.

Oral aureomycin therapy was begun on June 5, 4.0 g. being given during the first 24 hours and 2.0 g. per 24 hours for the succeeding 3½ days. On June 7, the patient felt considerably improved and his appetite was returning. The temperature became normal on the morning of June 8 and remained so thereafter.

Etiological data: Guinea pigs inoculated with a blood specimen taken on June 5 subsequently developed complement-fixing antibodies to *Coxiella burneti.*

Case 6 (FIGURE 5). G. S., male, 63, a slaughter-house worker, noted malaise and feverishness on May 29, 1948. During the following 4 days, he experienced repeated rigors, his temperature rose to 104° F. each evening and he complained of severe frontal headache, a non-productive cough, pains in the loins, and nausea. He was hospitalized on June 2. Physical examination at that time revealed an exceptionally robust man, who did not appear severely ill, but who was uncomfortable and perspiring profusely. Harsh breath sounds were noted over the left base posteriorly. The spleen was felt 1 fingerbreadth below the costal margin, and was firm and not tender. The white blood cell count was 5600. Roentgenograms of the chest failed to show any definite area of increased density.

Oral aureomycin therapy was begun on June 2, 4.0 g. being given during the first 24 hours and 2.0 g. daily thereafter for 3 days. During this period, marked symptomatic improvement was noted, appetite returned, and the patient's temperature returned to normal. His only complaints were a sore throat, soreness and a numb sensation referred

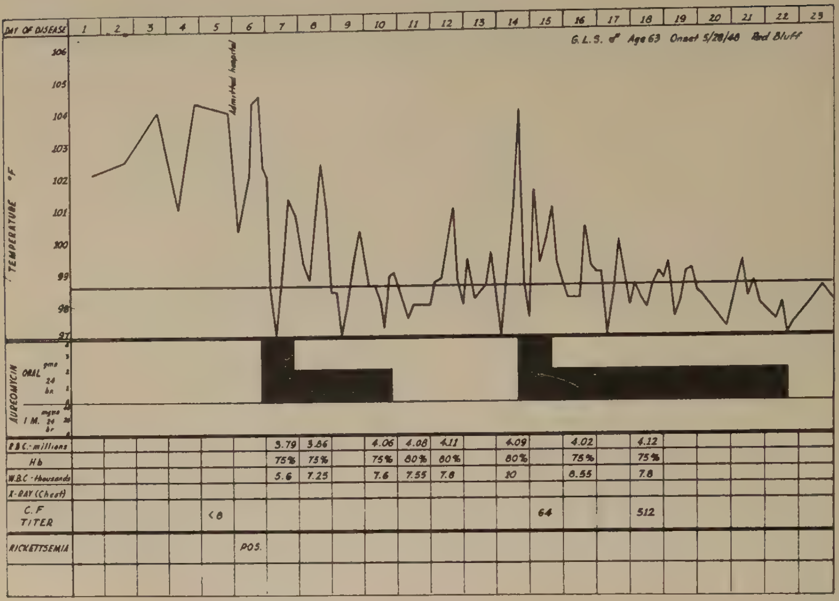

FIGURE 5. Relapse following cessation of therapy, and course after resumption of aureomycin treatment (Case 6).

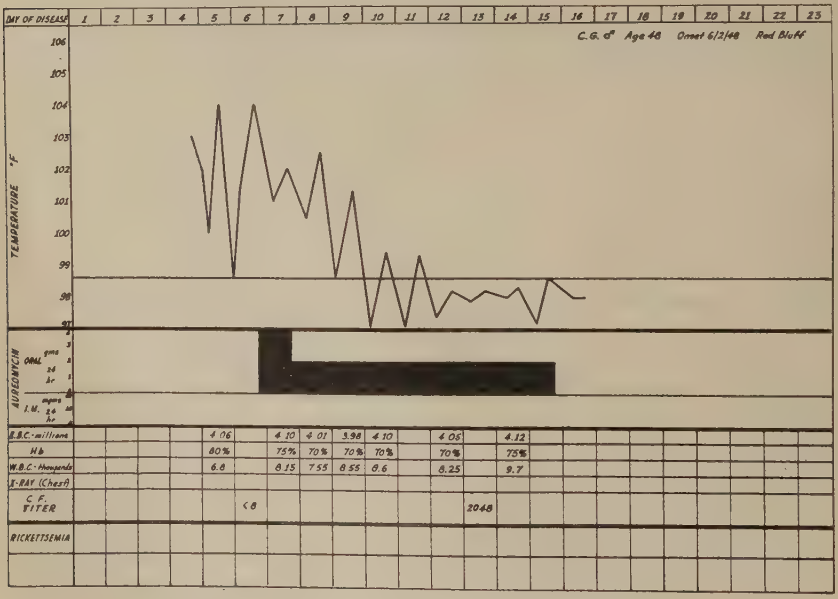

FIGURE 6. Course in patient treated with aureomycin (Case 7).

to the tongue, and a burning sensation on the surface of the scrotum. The tongue appeared smooth on the edges and was fiery red, as was the pharynx, and the scrotum presented a red, beefy appearance.

Following cessation of therapy, the patient's temperature remained normal for one day, but thereafter he began to have spikes of fever and on June 10 his temperature again reached 104° F. Oral aureomycin was resumed on the same schedule as before, and his temperature fell to normal during the succeeding three days. The drug was continued until June 18, a total of 17.5 gm. being given during this second course. The patient again noted some soreness and numbness of his tongue, but this tended to lessen during the period of therapy. His temperature remained normal following discharge from the hospital, and convalescence was uneventful.

Etiological data: Complement fixation tests for Q fever gave the following results: Titer, June 1, <1:8; June 11, 1:64; June 14, 1:512.

Case 7 (FIGURE 6). C. G., male, 48, a slaughter-house worker, became ill on June 2, 1948. He was admitted to the hospital on June 5 with complaints of malaise, feverishness, chills, headache, soreness of the eyeballs, generalized aching, anorexia and nausea. He coughed occasionally, raising small amounts of sputum. He had had severe shaking chills on 3 occasions. The white blood cell count was 6800. He was given penicillin, 30,000 units intramuscularly, every 3 hours for 3 days, but failed to show improvement.

When examined on June 8, his temperature was 102° F., pulse 76, respirations 80. He was a well-developed man who appeared moderately ill and uncomfortable. Fine *râles* were heard in the right posterior axillary region and the breath sounds were harsh in that area. The liver edge was felt 3 fingerbreadths below the costal margin and the liver was tender. The spleen edge was felt 1 fingerbreadth below the costal margin and was tender. Other physical findings were not abnormal.

Oral aureomycin was started on June 8, 4.0 g. being given during the first 24 hours and 2.0 g. each 24 hours thereafter, during the following 5 days, a total of 14.0 g. On June 10, the patient was more comfortable and his appetite had returned. The spleen was not felt, but the liver remained enlarged and tender. He was afebrile from June 13 on. Convalescence was uneventful.

Etiological data: On June 7, the patient's serum had a complement-fixing titer of <1:8, and on June 15, a titer of 1:2048.

Case 8 (FIGURE 7). F. M., male, 65, a railway shipping clerk, became ill on June 2, 1948, complaining of headache, pains in the loins, feverishness, repeated chills, nausea, and occasional vomiting. On June 9, following a shaking chill which lasted for two hours, his temperature rose to 104° F. On admission to the hospital at that time, he appeared moderately ill and fatigued. Physical examination failed to reveal any significant findings. White blood cell count was 5000.

He was placed on oral aureomycin therapy, 4.0 g. being given during

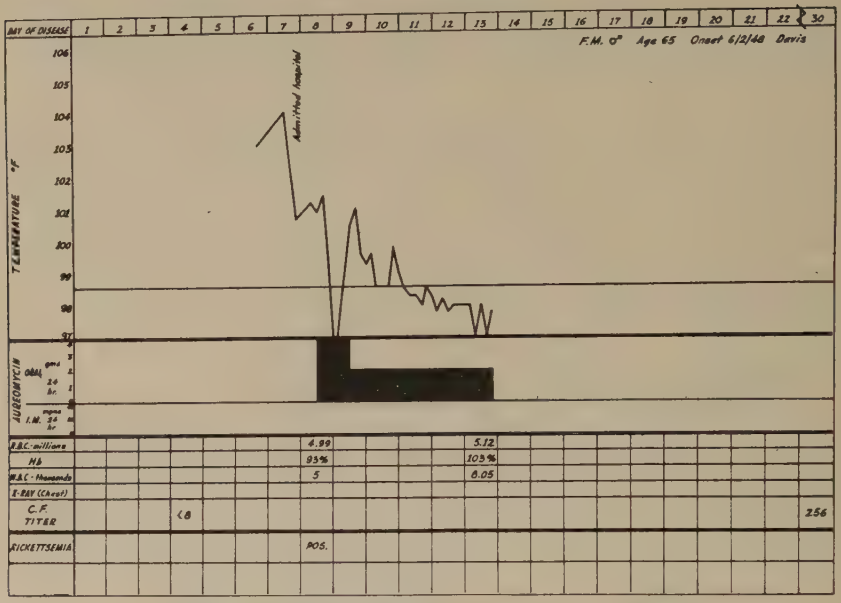

FIGURE 7. Course in patient treated with aureomycin (Case 8).

the first 24 hours and 2.0 g. daily thereafter during the following 4 days. The total dose of drug given was 12.0 g. Considerable symptomatic improvement was noted on June 10 and his temperature returned to normal on June 12. Convalescence was uneventful.

Etiological data: The Q fever complement fixation titer on June 5 was <1:8 and July 1 was 1:256.

Chronic Cases. Oral therapy has been used in 5 patients, mentioned above, who ran febrile courses of from 23 to 77 days. Four of these appeared to respond promptly. The fifth, a man of 51 years of age, who had been ill for 27 days when therapy was commenced, had failed to show, at the time of writing, appreciable response even with very large doses.

Toxicity

Mild symptoms referable to the gastro-intestinal tract were recorded in four cases. The chief complaints were a sense of fullness in the epigastrium with nausea and, occasionally, vomiting. Since all of these patients had had similar complaints prior to the beginning of therapy, the role of the drug in producing them is difficult to assess. One patient complained of pruritus and soreness of the scrotum and of soreness of the mouth. Both organs appeared inflamed. A second patient had similar complaints referable to the scrotum and also developed a small number of pruritic papular lesions over the shoulders. In both instances the drug was continued and symptoms did not become increasingly troublesome.

No evidence of hematological changes, of changes in urinary sediment, or of drug fever was observed.

Discussion

The need for an effective therapeutic agent for Q fever has become a very real one in California. It is still premature, however, to state categorically that aureomycin will meet this need, since the number of cases which it has been possible to treat during a 2-month period under conditions permitting adequate study is still relatively small, and larger numbers are needed to establish the effectiveness of the drug in a disease which varies considerably in severity and duration, and apparently is rarely fatal.

At the present time, the drug appears to have had a definite therapeutic effect. First, in a number of exceptionally ill patients, symptomatic improvement, frequently striking, has been observed in virtually all instances soon after therapy was begun. Secondly, the duration of fever has been, with few exceptions, short, and the number of patients treated is sufficiently large to make it seem unlikely that this drop in temperature has been due to chance.

It should be noted that there have been some obvious differences in the rapidity with which patients have responded to treatment. It is not yet clear what factors are responsible for these differences. Under consideration are such factors as the stage of the disease at which therapy was begun and the failure of the patient to obtain sufficiently high blood levels of the drug. It may be more than coincidental that the poorest therapeutic responses and the single therapeutic failure observed to date have occurred in patients with nausea and vomiting, and in whom aureomycin blood levels have tended to be low. Much work is needed to correlate dose, whether oral or parenteral, with blood level, and to determine what levels need to be obtained. The possibility that certain rickettsial strains are more resistant to, or may acquire resistance to, the drug also requires investigation.

The occurrence of relapses in 2 patients after therapy was stopped, together with the relatively slow response in others, suggests that, in the dosages used, the effect of the drug may have been to suppress rather than to eradicate the infecting agent. Unless it is found that larger doses of the drug have a much more prompt effect on the course of the disease, it would thus appear that recovery depends on the development by the patient of sufficient immunity to overcome the infection while the drug is exerting a suppressive action. In this series, there has been one instance, in a patient treated on the second day, in which the development of complement-fixing antibodies appeared to have been lessened by the use of the drug.

In the present study, evidence of drug toxicity has been sufficiently slight to justify the use of still larger amounts of aureomycin in the treatment of future cases. Such a procedure may make it possible to obtain a more uniform and more prompt response.

Summary

1. Results of aureomycin therapy of 19 Q fever patients are presented.

2. The results in a group of 4 patients treated by the intramuscular route with small doses were not considered satisfactory.

3. Of the remaining 15 patients, all treated orally, improvement occurred in 14 relatively promptly after commencement of therapy. The fifteenth patient, classified as a chronic case of Q fever, failed to respond even to large doses of the drug.

4. Relapses occurred in 2 patients in the orally-treated group following cessation of therapy. However, both patients became, and remained, afebrile following a second course of aureomycin.

5. It is concluded that oral aureomycin therapy offers considerable promise in the treatment of Q fever and that further investigations are desirable to evaluate the usefulness of the drug.

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